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THE SURGICAL RELIEF OF SEVERE ANGINA PECTORIS*

METHODS EMPLOYED AND END RESULTS IN 83 PATIENTS**

JAMES C. WHITE, M.D. AND EDWARD F. BLAND, M.D.

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I. INTRODUCTION

This report is a record of the experience at the Massachusetts General Hospital with surgical measures for relief of intractable angina pectoris during the past twenty years. It includes an historical account of the development of present day knowledge of cardiac innervation, a critical appraisal of the operative procedures available, with a summary of the results in 83 instances, and recommendations for the selection of patients suitable for this form of therapy.

To François-Franck (1), professor of physiology in Paris in 1899, is accredited the suggestion that sympathectomy would relieve the pain of angina pectoris. In spite of the intense suffering of those afflicted with an inadequate coronary circulation who fail to respond to medical treatment, surgeons were slow to put this idea to the test and cardiologists reluctant to adopt it. This conservative attitude was due in large part to Sir James Mackenzie's (2) dictum that it would be unfortunate if surgeons should discover an operation to relieve the pain of angina pectoris, because it would remove the vital danger signal which warns the patient when he is overtaxing his heart. It has long since been shown that this is not the case, but even to day the average internist's fears on this score have not been completely dissipated.

The first such sympathectomy was performed in 1916 by Thomas Jonnesco (3) with brilliant success. The Roumanian surgeon removed the chain of cervical sympathetic ganglia which gave rise to the three then known cardiac

* Presented during the Symposium on Cardiac Disease at the Centennial Celebration of the University of Buffalo Medical School October 1, 1946

** From the Neurosurgical Service and Cardiac Clinic of the Massachusetts General Hospital, Boston, and the Surgical Research Laboratories of the Harvard Medical School at the Massachusetts General Hospital

nerves (Fig. 1). During the next decade many modifications of cervical sympathectomy were described and put to the test with more or less disappointing results and a mortality rate of over 20 per cent. The literature of these earlier surgical endeavors has been reviewed by Fontaine (4) and Cutler (5). These

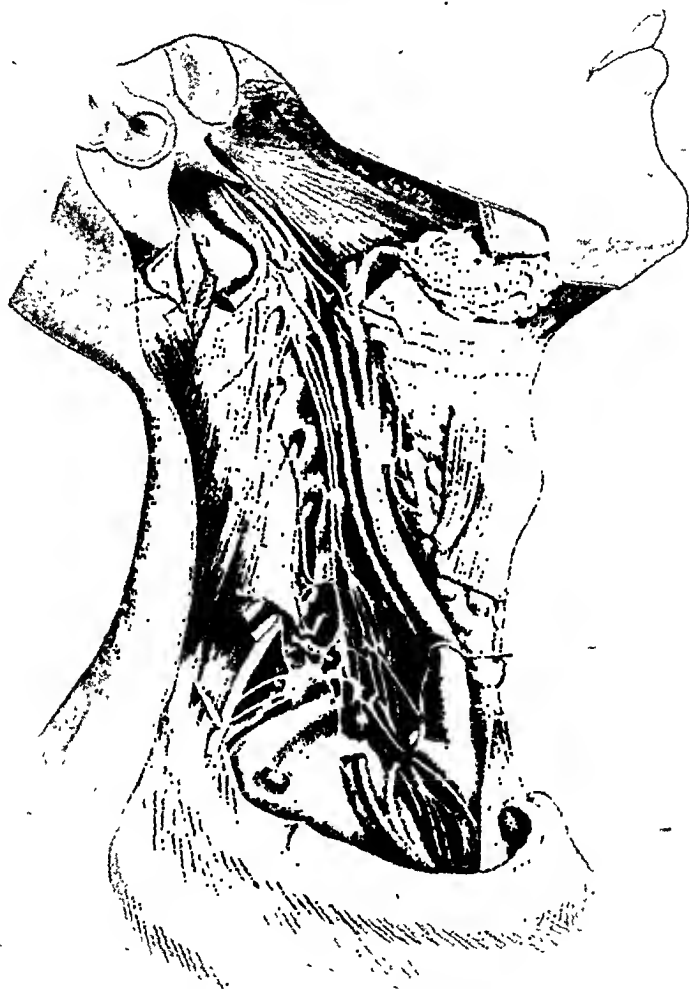


FIG. 1. THE CERVICAL CARDIAC NERVES, AS ILLUSTRATED BY JONNESCO

The superior, middle, and inferior cardiac nerves are shown running from the corresponding sympathetic ganglia downwards into the mediastinum. (From T. Jonnesco, "Le sympathique cervico-thoracique," Paris, Masson & Cie., 1923.)

statistics are summarized in Table I. At the Massachusetts General Hospital the operative statistics of our predecessors, Drs. C. A. Porter and E. P. Richardson, were even more disappointing. When surgical denervation relieves less than two patients out of three of their pain, it is obvious that important nerve pathways are being missed. Such was the situation in 1927 when we started a search for more effective surgical methods.

The stimulus which led to this study was the publication by Mandl (6) and

Swetlow (7) of the promising results of infiltration of the upper thoracic sympathetic ganglia and rami with procaine and alcohol. Thanks to the interest and cooperation of Dr. P. D. White, an opportunity was given to test Swetlow's method of paravertebral injection with alcohol and the results were found to be encouraging (8). At about the same time anatomists (9, 10, 11) first demonstrated the presence of definite rami from these upper thoracic ganglia running in the direction of the heart. How many of these fine fibres reached the cardiac plexuses and how many terminated in the hilus of the lung could not be ascertained by dissecting room methods. In order to make sure of the presence of lower cardiac nerves, it was necessary to find out how many of the thoracic ganglia and rami would have to be resected in order to interrupt all sensation of experimentally induced pain in animals.

TABLE I
Results of Cervical Sympathectomy in Angina Pectoris

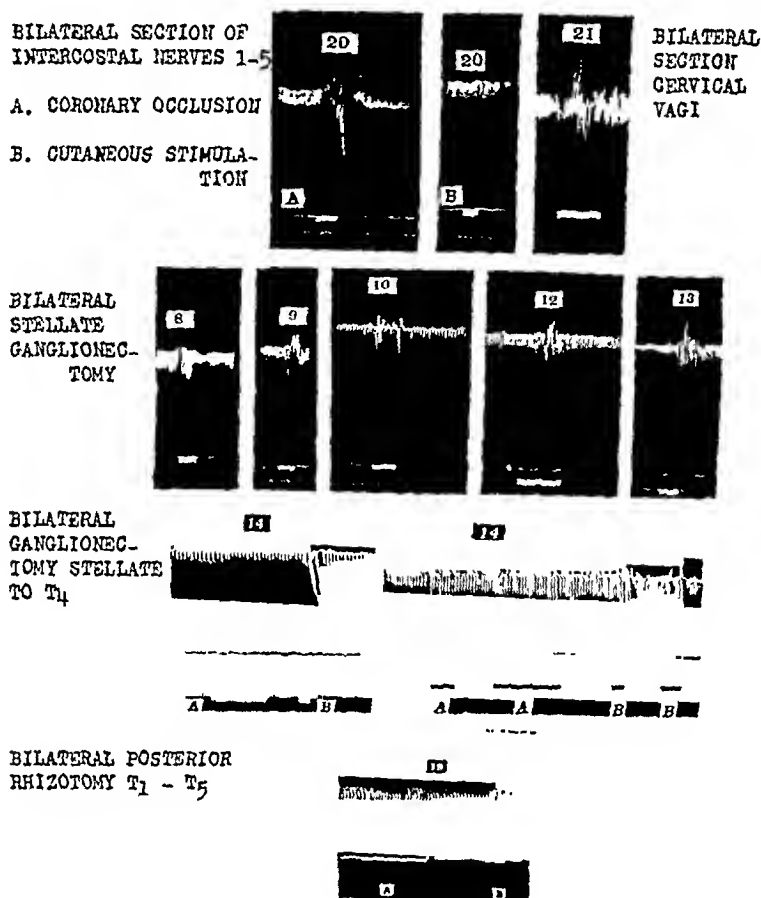
	UPPER CERVICAL SYMPATHETOMY			COMPLETE CERVICAL SYMPATHETOMY OR STELLATE GANGLIOECTOMY		
	Fontaine	Cutler*	M. G. H. **	Fontaine	Cutler*	M. G. H. **
Number of cases*	57	53	8	37	27	2
Results:						
Good	66.0%	41.5%	37.5%	56.8%	52.0%	50%
Improved..	12.3%	35.8%		10.8%	18.5%	
Failures	5.3%	11.2%	50.0%	8.1%	7.5%	
Uncertain	5.3%	4.0%		5.4%		
Deaths (within 4 days)	10.5%	7.5%	12.5%	18.9%	22.0%	50%

* Cutler's figures for mortality have been corrected to include deaths occurring within the first four days after operation.

** Massachusetts General Hospital cases operated upon by Drs. C. A. Porter and E. P. Richardson.

The heart, like the abdominal viscera, had first been thought to be devoid of pain sensation. It is of interest to find that early observations on this subject date all the way back to William Harvey (12). His subject was the young son of Count Montgomery, a friend of King Charles I, who had received a severe wound in the chest as a child. Although the thoracic cavity had been opened widely, the accident had not ended in death but in healing with the heart exposed. On taking off a sort of cardiac cuirass, Harvey saw the beating heart. Touching, pricking, or pinching the heart caused not the slightest sensation. A similar modern observation has been put on record by Alexander, MacLeod, and Barker (13), who studied a patient with open drainage of the pericardium which exposed the lower portions of the ventricles and the diaphragm. They also found the visceral pericardium over the ventricles to be insensitive to touch. Heat, cold, and vibrations were not perceived at all, and electrical stimulation evoked sensation only when it produced extra systoles.

These observations are in line with Lennander's (14) findings that the viscera are insensitive to cutting, crushing, and even burning. The error in their interpretation lay in the failure of these early experimenters to utilize a proper physiological stimulus. In the case of hollow viscera, Hurst (15) has shown that



EXPERIMENTAL CARDIAC PAIN IN DOGS

FIG. 2. EFFECT OF VARIOUS NEUROSURGICAL PROCEDURES ON EXPERIMENTAL CARDIAC PAIN IN DOGS

(See White, Garrey, and Atkins (19)). The upper tracing in each figure represents the respiratory excursion. The signal in the lower line represents periods of occlusion of the descending branch of the left coronary artery. At signals marked B in the two lower tracings the toe pads were pinched. Whenever respiratory changes occurred the animals showed clear-cut evidence of discomfort.

distension is the physiological stimulus, while for the heart the more recent work of Sutton and Lueth (16) and others (17, 18) points to myocardial anoxia and abnormal products of muscular fatigue as the cause of angina pectoris. The publication of Sutton and Lueth's method of inducing coronary pain in dogs by temporary occlusion of the descending branch of the left coronary artery* permitted White, Garrey, and Atkins (19) to carry out the necessary

* In using Sutton and Lueth's preparation, a silk ligature was passed beneath the upper portion of the descending branch of the left coronary artery and then brought out through

crucial experiments. This work, published in 1933, showed that myocardial ischaemia was still productive of pain after bilateral cervical sympathectomy carried down through the stellate ganglia (Fig. 2). On the other hand, no pain could be induced, even by prolonged coronary occlusion, after resection of both stellates and the upper four pairs of thoracic ganglia or division of the upper five pairs of thoracic posterior spinal roots. This experiment gave the necessary physiological proof of the presence of direct thoracic cardiac connections. Whether the anatomical arrangement was the same in man as in the dog still remained to be proved. Results of chemical and surgical deafferentiation of the heart have long since proven that a very similar anatomical arrangement exists in man.

II. NEUROANATOMY AND PHYSIOLOGY

Study of Figure 3 will serve to illustrate the most practicable methods for achieving a complete interruption of pain-conducting pathways from the heart. Sensory axones from the cardiac plexuses reach the chain of paravertebral ganglia over the middle and inferior cardiac nerves, and also over the upper thoracic rami. The great majority, if not all, of these enter between the middle cervical and third thoracic ganglia on both sides of the chest. Evidence has been presented by Pollock and Davis (20) that the superior cardiac nerve and upper cervical ganglion from which it arises contain no afferent connections with the spinal cord. There is no anatomical explanation for the early reports (Coffey and Brown (21)) of occasional successful results of superior cervical gangliectomy in the relief of angina pectoris. It may be pointed out, however, that favourable responses have been so inconsistent that this operation has no present-day advocate.

The lower level at which sensory impulses enter the paravertebral chains is at the third thoracic ganglion in the great majority of cases (see protocol of Case 6B below). Although a cardiac ramus is shown in Figure 3 joining the fourth thoracic ganglion, its existence must be questionable, except in the rare instance of a "post-fixed" arrangement of the cardiac outflow. Central connections between the paravertebral ganglionated chains and the spinal cord are all concentrated in the upper thoracic region and reach the posterior horn of grey matter over the upper four posterior thoracic roots. There are no direct central connections between the middle and inferior cervical ganglia and the cord, because there are no connecting white rami communicantes in the cervical portion of the chain. Impulses which enter the cervical ganglia over the middle and inferior cardiac nerves must, therefore, all descend to the upper thoracic level before they can cross in the white rami to join the thoracic spinal nerves.

the chest wall in a glass tube. These animals suffered no shock and recovered rapidly from ether anaesthesia. They were then ready for testing within a few hours, the type of cardiac denervation to be evaluated having been carried out several days previously. When cardiac pain fibres remained uncut, traction on the ligature for a period of only a few seconds invariably produced a change in the rate and depth of respiration, soon followed by unmistakable evidence of discomfort. The stimulus was always interrupted before any real suffering was produced.

It is, then, obvious that all afferent pathways are most conveniently concentrated in the upper thoracic ganglia and posterior spinal roots. In concluding this anatomical description of the cardiac nerves, it is of fundamental importance to emphasize the simplicity of this central arrangement and its ever-increasing complexity in the periphery.

Surgical experience based on resection or effective chemical block of the upper three thoracic ganglia serves to support the evidence outlined above, as these

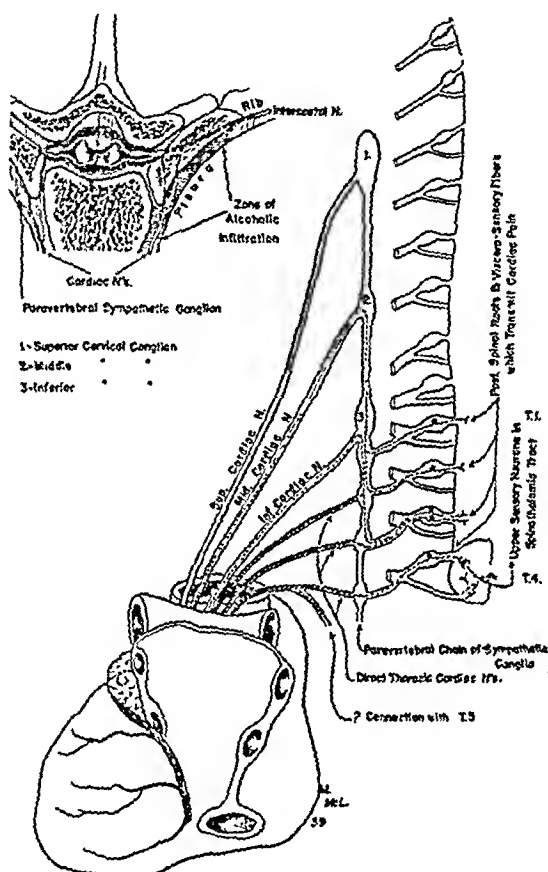


FIG. 3. THE SENSORY NERVES OF THE HEART

Although a ramus is shown connecting the fourth thoracic ganglion with the heart, its occurrence is rare, and the presence of any still lower connections is unlikely. (From article by White (29), courtesy of *Surgery, Gynecology, and Obstetrics*.)

procedures have given consistent relief of pain referred to the arm and precordial areas. Posterior rhizotomy with division of the four thoracic sensory roots has proved to be an equally effective operation.

The only area to which we are aware that pain can be referred after these crucial lines of communication have been destroyed is the lower jaw and ear. This unusual radiation has been seen in 3 patients, and in 1 case still persisted after resection of the superior cervical ganglion and the superficial cervical plexus. Olivecrona (22) has also observed this and found that this pain disappeared after injection of the mandibular nerve. It is probable that its afferent

pathway to the brain stem is over the vagi. These nerves fortunately play no other role in the conduction of cardiac pain, although they probably transmit the dull sense of oppression felt in the suprasternal notch after complete sympathetic denervation of the heart.

Distribution of the cardiac sensory nerves is entirely unilateral, so that after their effective interruption on one side pain may still be present on the other, although this sensation stops at the midline. After bilateral interruption all sensation of pain disappears, but the patient will still appreciate when he is overtaxing his heart by a sense of painless oppression or constriction, usually located in the suprasternal notch (presumably transmitted by the vagi), or by dyspnoea, palpitation, and other sensations related to abnormal action of the heart. We have seen this essential danger signal lost temporarily in only a single instance. Here, after an unusually wide-spread paravertebral block with procaine, the patient, after taking an over-vigorous exercise tolerance test, developed an attack of intense but painless dyspnoea. In this instance it is reasonable to assume that the procaine had infiltrated the vagi as well as the sympathetic fibres to the heart.

From an anatomical and physiological point of view it is important to emphasize that cardiac and other visceral afferent fibres do not, strictly speaking, belong to the autonomic nervous system. The latter, by definition, is only concerned with glandular secretion, smooth muscle, and visceral activity. It should be borne in mind, however, that the nerves to the internal organs are mixed nerves, carrying sympathetic motor and somatic pain fibres. The latter differ from the former in having their cells of origin in the posterior root ganglia. Their central processes enter the posterior horn of grey matter and the peripheral fibres run in continuity through the paravertebral ganglia and cardiac nerves to the heart.

The mechanism of perception of cardiac pain is also of considerable physiological interest. Mackenzie (2) believed that it was entirely a referred phenomenon and that the bombardment of the posterior horn of grey matter in the cord by impulses from the diseased viscus lowered synaptic resistance to the point where normally sub-threshold stimuli from the surface of the body, entering the same spinal segments, became augmented to the level of conscious pain. This theory of Mackenzie's is now known to give an incomplete account of the phenomenon of visceral pain. Ross (23) and Head (24), in earlier theories, postulated both direct conduction of pain from a viscus—a deep, aching, poorly localized sensation (the ill-defined sense of oppression in the precordium)—and the more accurately localized referred sensation (pain in the arm). Weiss and Davis (25) have shown that, no matter how wide an area of superficial sensation is blocked by subcutaneous procaine infiltration, the pain is likely to appear at the periphery if the stimulus is sufficiently great. These investigators also admit that a dull unpleasant sensation may persist in spite of wide cutaneous anaesthesia. It is therefore far more practical to interrupt the visceral afferent nerves than to attempt to produce a permanent cutaneous anaesthesia of the entire area to which pain may be referred.

The anatomical concepts described above have been thoroughly tested by surgical experiences of the past decade. Referring again to Figure 3, it is obvious that the sensory cardiac fibres are most concentrated and accessible to the surgeon in the upper thoracic posterior spinal roots and in the corresponding thoracic sympathetic ganglia and rami. It is a general principle of sympathetic surgery that neurectomy can be carried out most effectively at the points where the complex peripheral plexuses are simplified in the paravertebral chains and spinal roots, and that peripheral operations, such as dissection of the plexuses along the coronary arteries, as recently proposed by Fauteux (26), are illogical from an anatomical point of view.

III. SENSORY DENERVATION OF THE HEART

Anatomical considerations, as pointed out in the preceding section, favor surgical intervention either on the upper thoracic paravertebral ganglia or on the corresponding posterior spinal roots, which carry the pain-transmitting fibres from the heart. These areas constitute the bottle-neck through which all pain-conducting axones from the widely ramifying peripheral plexuses are funneled before entering the spinal cord. When operating in this region the surgeon encounters structures which are easy to identify and free from unusual anatomical variations. Regeneration cannot take place after rhizotomy, and after resection of as extensive an area as the upper three thoracic ganglia it is most unlikely to occur.

This is not the place for a detailed outline of operative procedures, and surgeons who wish to familiarize themselves with the technical steps will find complete descriptions in previous papers (27, 28, 29, 30). We should like, however, to take this occasion to emphasize the minimal extent of each of the three standard procedures which can be utilized to insure a complete sensory denervation of the heart, and also to call attention to certain steps which add to their effectiveness or safety. The accompanying illustrations should help bring home these points.

1. *Chemical Block*

Paravertebral injection of procaine and 95 per cent ethyl alcohol at the sides of the upper four thoracic vertebrae was first proposed by Mandl (6) and Swetlow (7). Because the point of attack was shifted from the cervical region caudally to include the thoracic cardiac nerves, it became the first effective method for interrupting the entire complex of fibres which transmit pain from the heart. We shall, therefore, discuss it first.

The technique, with safeguards against infiltration of the pleura and subarachnoid space, has been perfected by one of us (J. C. W. (29)) and far greater accuracy in the placement of the needles assured by the use of x-ray control (30). (See Figs. 4, 5, and 6.) Patients who have had frequent and recent attacks of coronary thrombosis are poor risks for anaesthesia and any form of open surgical intervention. For these paravertebral injection of alcohol is the least dangerous method of interrupting the pathways of cardiac pain. We have seen patients

undergo injection without complications and then succumb to a later operation (see protocol in Cases 30A and 48A below).

There are, however, serious objections to chemical blocking of the cardiac nerves. In the most skilled hands failure to interrupt a sufficient proportion of the afferent fibres occurs in some 10 per cent of cases. Even with this procedure there is an appreciable risk of mortality in the most advanced cases. The principal danger lies in the fact that injection must be carried out without anaesthesia in order to observe the development of paralysis of the upper thoracic sympathetic outflow by the appearance of a Horner's sign and a hot, dry hand. The mild degree of discomfort and accompanying nervous strain have been sufficient to precipitate fatal coronary infarction in 3 of our patients. Two other men died in a similar fashion but, fortunately for us, a few hours before the

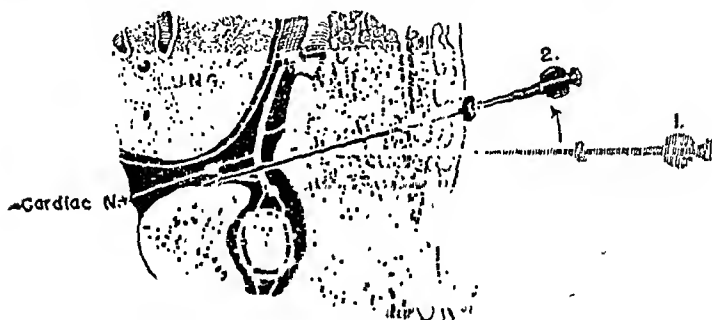


FIG. 4. INSERTION OF NEEDLE FOR INJECTION OF CARDIAC NERVES

1, Needle inserted 4 cm. to left of spinous process and tip in contact with transverse process of vertebra. Depth marker has been set at a point 3 cm. from the skin. 2, Shank of needle has been rotated outward and tip worked inward until at an additional depth of 3 cm. it lies in contact with the side of the vertebra and in close approximation with the ganglionated sympathetic chain. (From article by White (29), courtesy of *Surgery, Gynecology, and Obstetrics*.)

injection was scheduled. The statement is attributed to John Hunter (Home (31)) that his life was in the hands of anyone who might make him lose his temper. This prophecy was fulfilled, as he died at a medical meeting after being provoked by one of his colleagues. In this critical group fear of being hurt is unfortunately an equally dangerous form of psychic trauma. Adequate preliminary sedation of the patient serves to minimize this risk, but does not abolish it altogether. Another danger, which can be avoided, is injury to the spinal cord if the alcohol penetrates the subarachnoid space. This complication has fortunately not occurred in our series of 75 cases (many of which have had multiple injections) and we believe that it can be avoided if the recommended precautions are scrupulously followed. Myelitis has, however, been reported on 3 occasions (Molitch and Wilson (32), Olsen (33), and Hirschboeck and Gillespie (34)). Neuralgia due to irritation of the adjacent intercostal nerves is another less

serious problem. The sympathetic ganglia lie so close to the intercostal nerves that alcohol infiltrated around the chain cannot help bathing their trunks. This produces a certain amount of discomfort in nearly all cases and is a source of major complaint in some 10 per cent, but it can be counted on to clear up within a

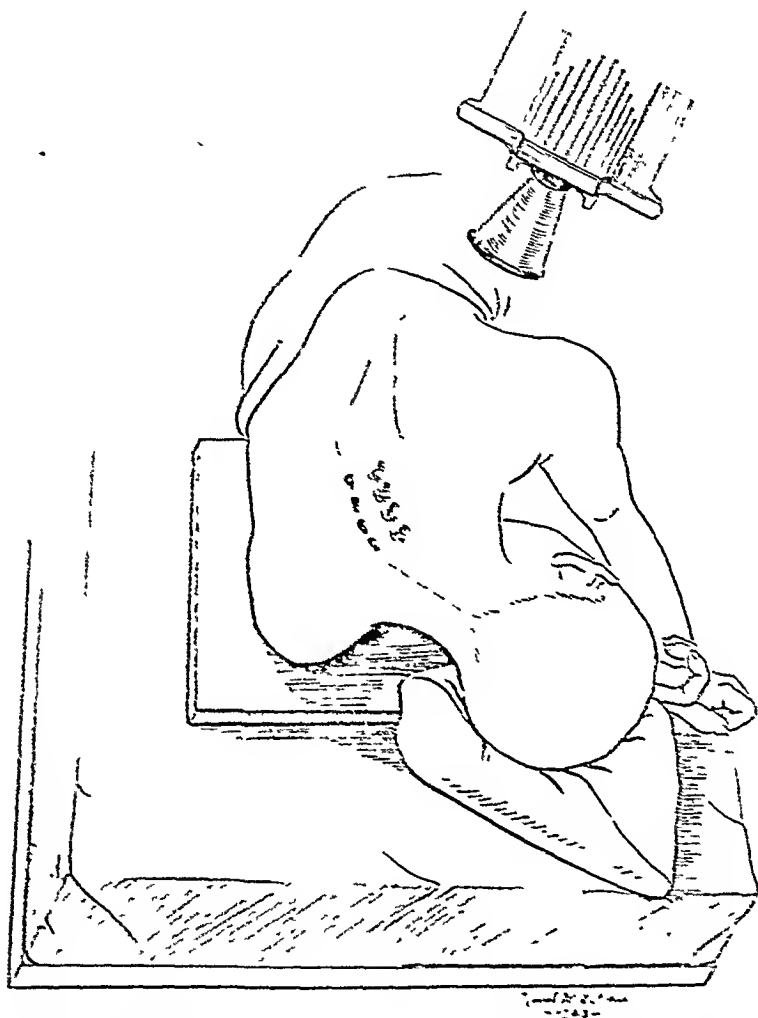


FIG. 5. DIAGRAMMATIC VIEW OF PATIENT IN BED WITH NEEDLES INSERTED AGAINST THE SIDES OF THE UPPER FOUR THORACIC VERTEBRAE, TO SHOW POSITION OF X-RAY TUBE AND CASSETTE

(From article by White and Gentry (30), courtesy of *Journal of Neurosurgery*)

month or two. A final point against the substitution of alcohol in place of surgical neurectomy is the fact that the block is not always permanent. Although 40 cases listed in Table II maintained their initial improvement for periods up to nine years, there was a recurrence of anginal pain after intervals of from two and one-half months to five years in 14 others. Judging from the temporary relief achieved by injection of alcohol in trigeminal neuralgia, it is surprising that the interruption of pain in angina pectoris has been of such long duration. With recent improvements in the technique of injection, and especially with radio-

logical localization of the needles prior to injection, failure to secure a satisfactory chemical block is certain to become less frequent



FIG 6 LATERAL X RAY TO SHOW POSITION OF NEEDLES TAKEN DURING AN ACTUAL PARAVERTEBRAL INJECTION

This patient developed a striking Horner's sign and a hot, dry hand, as proof of the effective impregnation of the upper ganglia. The tips of the two upper needles would have been in even closer contact with the ganglia if they had been inserted a centimeter deeper (From article by White and Gentry (30), courtesy of *Journal of Neurosurgery*)

In spite of these cogent objections, the relief of suffering and the general improvement seen in so many of our patients have convinced us that paravertebral injection with alcohol still has a valuable role in the treatment of the later stages of coronary heart disease with intractable angina pectoris. Mastery of the technique is well worth the effort required, because it enables so many otherwise hopeless sufferers to obtain relief from their pain.

Injection experiments in animals have shown that 5 c.c. of alcohol produces an area of necrosis not much over 1 cm. in diameter. Observations made during two postmortem examinations have shown that this likewise applies to man. The infiltration of alcohol must, therefore, be far more exact than when procaine is used, as the latter diffuses so much more widely through the retropleural tissues. It is always evident when the alcohol has been correctly placed, because of the unequivocal signs of paralysis of the upper thoracic sympathetic rami. When these signs persist, pain is as effectively relieved as though the same structures had been resected. Even if there is a gradual disappearance of

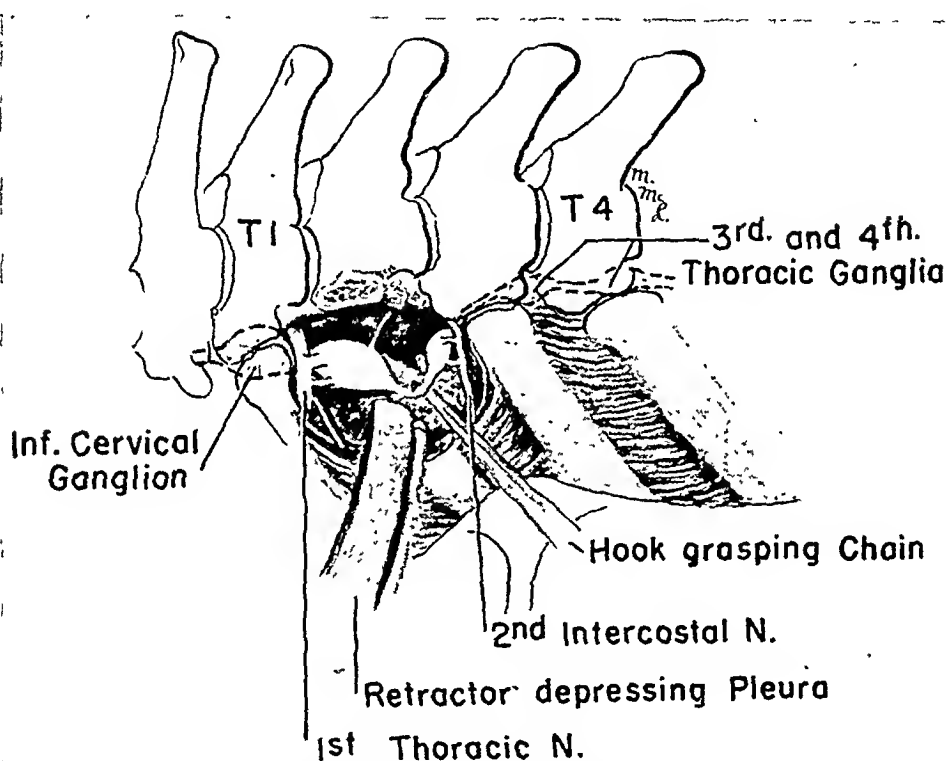


FIG. 7. DIAGRAMMATIC SKETCH OF EXPOSURE OF UPPER THORACIC SYMPATHETIC GANGLIA AFTER REMOVAL OF CENTRAL PORTION OF SECOND RIB AND RETRACTION OF PLEURA

the Horner's sign and a reduction of the initial vasomotor and sudomotor paralysis, regeneration of the cardio-sensory fibres is less likely to take place.

2. Sympathetic Ganglionectomy

This operation is best performed through the posterior approach with resection of the central end of the second rib and transverse process (see Fig. 7). Ether-oxygen anaesthesia is administered through an intratracheal catheter with the patient in the lateral position. White and Smithwick (27) formerly advocated use of the anterior approach, but we have since found that the route through the posterior portion of the second rib gives a far more direct exposure and permits the necessary extrapleural resection from the inferior cervical ganglion to a

point well below the third thoracic. When the inferior cervical and first thoracic components of the stellate ganglion are separate as a dumbbell-shaped structure removal of the lower portion is sufficient, but when fusion has taken place the entire ganglion should be resected. It is never necessary to carry the resection upwards into the cervical region, but it is essential to follow the chain downwards to include the third thoracic ganglion (see protocol on Case 6B below).^{*} The oblique incision advocated by White, Smithwick, Allen, and Mixter (35) is most useful, as it is a muscle-splitting incision and runs parallel to the normal planes of cleavage in the skin. In the hands of an experienced surgeon this operation can be done in under an hour, and with a minimal risk of postoperative complications.

The results have been excellent (see below). This has become the operation of choice at the Massachusetts General Hospital, where our surgeons have technical facility in this region from frequent operations in Raynaud's disease and hyperhidrosis. The only objection lies in the fact that if pain is bilateral or later appears on the unoperated side a second ganglionectomy may have to be performed on the opposite side. In addition, a sympathectomy at this level produces a Horner's sign with partial ptosis and myosis. Even so we feel that the bilateral two-stage operation is safer for the patient and therefore the preferable procedure. The single operative death in this series occurred seventeen years ago before the routine use of intratracheal anaesthesia and modern chemotherapeutic methods of treating postoperative pneumonia.

3. *Posterior Rhizotomy*

The effectiveness of this operation was first demonstrated in animal experiments by White, Garrey, and Atkins (19), but has not been developed further at the Massachusetts General Hospital because of the apparently groundless fear that it would be too much of an operation for the average patient with advanced coronary disease. This, fortunately, has not proved to be true. We have recently been able to obtain reports of 29 such operations from the literature, in which the results were consistently excellent (see below). There were 3 operative deaths.

These statistics show that the operation, performed under intratracheal administration of ether and oxygen, is a comparatively safe procedure and one nearly certain to put an end to all pain felt in the precordial area or referred to either arm. The minimal number of posterior roots which should be sectioned are the upper four thoracic on both sides (see Fig. 8). Its outstanding advantages are:

- 1) The technique of laminectomy and posterior root section is a standard operation and well understood by the average neurological surgeon.
- 2) There is no possible chance of regeneration.
- 3) The cardiac afferent fibres can be cut on both sides at a single operation.

^{*} Olivecrona (22) has been carrying the resection of the chain as low as the fourth or even the fifth ganglion for good measure, as he feels that this will prevent occasional persistent pain low in the precordium.

Although the patient often complains of severe angina pectoris on only one side, there is often more or less involvement of the opposite thoracic and arm areas once the worst pain has been relieved.

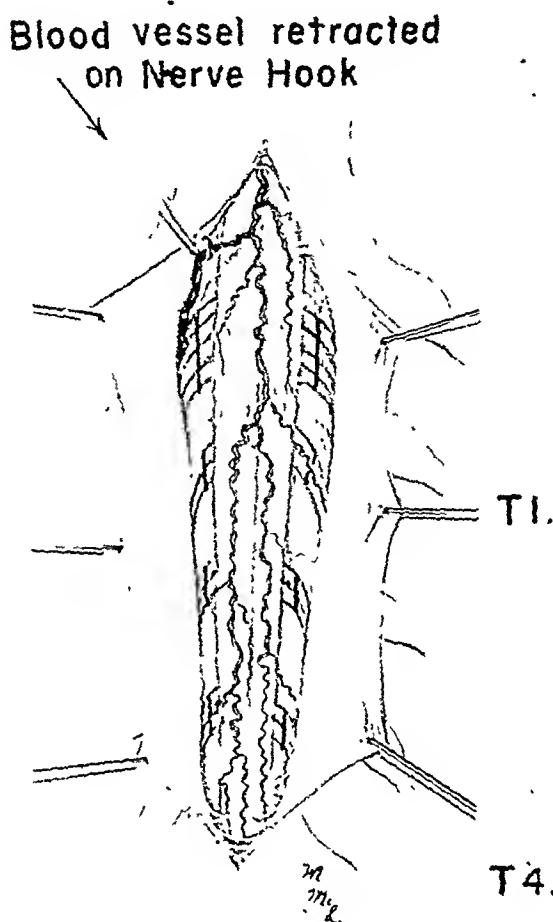


FIG. 8. DIAGRAMMATIC SKETCH OF APPEARANCE OF UPPER FOUR POSTERIOR SPINAL NERVE ROOTS AND THEIR POINT OF DIVISION FOR RELIEF OF CARDIAC PAIN

These advantages are counterbalanced to a certain extent by the fact that:

- 1) Laminectomy of four vertebrae is a time-consuming procedure.
- 2) Bilateral root section, even if the blood vessels which accompany the nerve roots are carefully preserved, involves the occasional risk of an ischaemic transverse myelitis. This tragic complication has been reported after extensive bilateral anterior root section for hypertension (Page and Heuer (36)), and it has occurred in a case of severe hypertension after posterior rhizotomy for atypical cardiac pain performed by Mixter and White in 1934.

- 3) Laminectomy is usually performed in the prone position. This position

limits normal inspiratory excursion, thereby reducing negative intrathoracic pressure and the return of blood to the right side of the heart. Even with careful attention to placement of the patient on the operating table, this is a distinct handicap, especially to an obese patient with coronary disease. It can be eliminated by performing the operation with the patient in the lateral position.

4. Selection of Cases

Surgical treatment of angina pectoris should be reserved for the group of patients who, after adequate observation, cannot be controlled effectively on a medical regime. There is no longer justification for permitting a patient to suffer from continued angina decubitus, with exhaustion from lack of sleep or constant worry over his next attack and the likelihood of gradual addiction to narcotic drugs. Under these circumstances surgical intervention may actually prolong life by the relief of pain during a particularly dangerous period of coronary insufficiency until the collateral circulation of the myocardium improves; but this latter is at best a slow evolution, often requiring months before significant results are evident. In numerous instances these patients have ultimately been able to return to work as their coronary circulation improved, with only mild restriction of activity and considerable reduction in their need for nitroglycerine.

The patient himself should be the final judge in the decision between continued dependence on drugs, with the necessary rigid limitation of his activity, and the more dangerous method of relieving his pain by surgery, with its more rewarding possibilities. He alone knows the exact degree of his discomfort and the mental disturbance induced by the fear of imminent death. It is therefore best to discuss the matter frankly with the patient and tell him the price he must pay in the way of risk and temporary discomfort in return for possible freedom from fear of pain and the likelihood of a considerable increase in his activity and enjoyment of life.

Once the decision has been made in favour of surgical intervention, the intelligent choice of the proper surgical procedure depends on the relative competency of the heart. Those with a fair cardiac reserve may be submitted to laminectomy and root section with reasonable safety. This operation is most logical when the reference of cardiac pain is bilateral, or when the surgeon is not experienced in the technique of sympathectomy. For the more questionable risks, thoracic ganglionectomy is preferred, especially if the anginal pain is unilateral. For the poorest risk cases, viz., those patients who have had repeated attacks of coronary infarction, with large hearts and great reduction in cardiac reserve, interruption of the nerves by paravertebral alcohol block may be the only possible recourse.

IV. CLINICAL RESULTS

1. Paravertebral Injection with Alcohol

The results in 75 patients in whom this procedure has been used at the Massachusetts General Hospital are summarized in Table II. Among the 56 per

TABLE II
Relief of Pain in Severe Angina Pectoris—by Paravertebral Alcohol Injection—75 Cases
 1. Excellent Results—42 Cases

NO.	PATIENT	AGE	ÆTIOLOGY	INJECTION	TEMPORARY NEURALGIA	ACTIVITY AFTER INJECTION	RECOVERY	SURVIVAL	CAUSE OF DEATH	PAIN AT DEATH
1A	William M.	54	Syphilitic aortitis with regurgitation, hypertension, and angina decubitus.	Bilateral	++	Intermittent congestive failure prevented return to work.	0	6½ yrs.	?	?
4A	*George B.	51	Coronary heart disease.	Left	+	Able to drive truck for 3 mos., then incapacitated by dyspnoea.	0	Alive at 2½ yrs.		
6A	Injection performed by Dr. W. J. Mixter	52	Hypertensive heart disease, aortic regurgitation, and angina decubitus.	Left	-	Light work.	0	Alive at 2½ yrs.		
9A	Injection performed by Dr. W. J. Mixter	47	Hypertensive and coronary heart disease, myocardial infarct (old), and angina decubitus.	Left	-	Limited by dyspnoea.	0	2 mos.	Congestive failure	Right side only.
11A	*Jasper H.	56	Hypertensive and coronary heart disease.	Left	+	Worked hard for 4 mos., then incapacitated by "stroke."	Arm pain after 4 mos.	22 mos.	?	?
12A	*Chris. D.	57	Hypertensive and coronary heart disease, myocardial infarct (old).	Left	+	Continues to do light work.	Partial after 1 yr.	Alive at 3½ yrs.		
13A	Dr. John H.	56	Coronary heart disease, myocardial infarct (old).	Left	+	Resumed active medical practice.	0	4 yrs.	Lymphoma.	0
15A	*Fred H.	49	Coronary heart disease.	Left	0	Worked 3½ yrs.	Partial after 2½ yrs.	3½ yrs.	Coronary thrombosis (autopsy).	Right side only.
18A	George M.	70	Hypertensive and coronary heart disease, aortic stenosis.	Left	0	Limited by dyspnoea.	0	Alive at 2 yrs.		
19A	*Laura P.	64	Coronary and hypertensive heart disease, myocardial infarct (old).	Right	+	Light housework.	0	Alive at 2½ yrs.		

21A	John L	43	Coronary and rheumatic heart disease, mitral stenosis	Left	+++	Limited by dyspnoea	Partial at 1½ yrs	22 mos	Operation on mitral valve (elsewhere)	0
22A	*Max R	59	Coronary heart disease	Left	++	Limited by mild right-sided angina pectoris	0	3½ yrs	Coronary thrombosis	Right side only
23A	H G T	53	Coronary and rheumatic heart disease, previous congestive failure myocardial infarct (old)	Left	0	Light work	0	4 mos	Coronary thrombosis and congestive failure	0
24A	Hyman J	64	Coronary heart disease, myocardial infarct (old)	Left	+	A professional strong man who did not return to work	5 yrs	7½ yrs	?	?
26A	Benj. G	61	Coronary heart disease, myocardial infarct (recent)	Left	0	Sedentary	0	2 mos	Acute coronary insufficiency	0
27A	*Jeanne L	60	Coronary heart disease	Left	+	Sedentary	0	Alive at 9 mos		
28A	Maybourns W	54	Coronary disease with myocardial infarct (recent), angina decubitus	Left	+	Continues to do light work	Slight at 5 yrs	Alive at 7 yrs		
29A	Dr Wm N	53	Coronary disease with myocardial infarct (old and recent)	Left	+	Returned to medical practice, re-entry later for right-sided block	0	14 mos	Coronary thrombosis on day scheduled for right block (autopsy)	Right side only
30A	Dr Paul H	57	Coronary disease and status anginosus	Left	++	Resumed medical practice (U S Army)	0	8 mos	Acute coronary insufficiency	0
35A	Evelyn C	24	Rheumatic heart disease with aortic and mitral regurgitation and stenosis, angina decubitus	Left	0	Worked as seamstress	0	4 yrs	Subacute bacterial endocarditis	0
37A	Charles J	17	Rheumatic heart disease, aortic and mitral regurgitation and stenosis, angina decubitus	Bilateral	0	Limited by dyspnoea	0	7 mos	Congestive failure (autopsy)	0

TABLE II—Continued

NO.	PATIENT	AGE	ETIOLOGY	INJECTION	TEMPORARY NEURALGIA	ACTIVITY AFTER INJECTION	RECOVERY	SURVIVAL	CAUSE OF DEATH	PAIN AT DEATH
38A	•Dr. G. W.	40	Coronary disease.	Right	++	Resumed limited medical practice. Mild left-sided attacks continued.	0	3 yrs.	Acute coronary insufficiency.	+
39A	James I.	63	Coronary disease with angina decubitus.	Bilateral	0	Resumed work in store.	0	8 mos.	Coagulative failure after gall bladder operation.	0
41A	Rebecca G.	53	Coronary disease and aortic stenosis. Diabetes mellitus. Angina decubitus.	Left	+	Light housework, rare need for nitroglycerina.	0	Alive at 6 mos.		
43A	Abigail W.	60	Coronary heart disease and paroxysmal auricular fibrillation.	Bilateral	+	Moderate housework.	6 mos.	Alive at 2 yrs.		
45A	Chris. P.	51	Syphilitic heart disease with aortic regurgitation, angina decubitus.	Left	0	Light work.	0	6 mos.	Acute coronary insufficiency.	?
46A	•Abraham G.	58	Coronary and hypertensive heart disease, diabetes mellitus.	Right	+	Worked briefly as salesman until cerebral thrombosis.	2½ mos.	Alive at 10 mos.		
48A	•Julia T.	65	Coronary and hypertensive heart disease.	Left	+++	Limited by dyspnoea.	2½ yrs.	Alive at 4 yrs.		
51A	Amelia F.	60	Coronary and hypertensive heart disease, myocardial infarct (old), angina decubitus.	Left	+	Active, useful life.	0	Alive at 9½ yrs.		
54A	Ernest H.	52	Syphilitic and coronary heart disease.	Left	0	Limited by dyspnoea and right-sided angina.	0	6 mos.	Acute coronary insufficiency.	Right side only.
56A	•Frank C.	62	Coronary and hypertensive heart disease.	Left	++	Moderate activity but continued right-sided angina peccatoris.	0	6½ mos.	Coronary thrombosis.	Right side only.

59A	*Lily M.	54	Coronary, hypertensive, and rheumatic heart disease, mitral stenosis.	Left	+++	Moderate, at home.	2 yrs.	Alive at 2 yrs.		
61A	*Reginald F.	46	Coronary heart disease, myocardial infarct (old).	Bilateral	+++	Active up to death.	0	4 yrs.	Drowned at sea while yachting.	0
62A	Dr. Chas. S.	54	Rheumatic heart disease, aortic regurgitation.	Bilateral	++	Limited by dyspnoea.	Partial, left, at 5 mos.	1 yr.	Acute coronary insufficiency.	?
63A	Dr. George M.	79	Coronary heart disease with aortic stenosis and carcinoma of prostate.	Left	++	Residual attacks in arm, activity limited by advanced carcinoma.	Partial, after several mos.	7 mos.	Uncertain.	?
64A	John W.	72	Coronary and hypertensive heart disease, angina decubitus.	Right	0	Limited by dyspnoea and mild right-sided angina.	0	2 yrs.	Coronary thrombosis(?)	?
65A	Camillo W.	62	Coronary heart disease, diabetes mellitus, angina decubitus.	Left	++	Sedentary.	0	2½ mos.	Cause unknown.	?
67A	John M.	70	Coronary and hypertensive heart disease, myocardial infarct (old). Incapacitated 6 yrs.	Left	0	Almost normal. Worked to day of death. Practically no need for nitroglycerine.	0	3 yrs.	Acute coronary insufficiency.	+
69A	*Nathan N.	61	Coronary and hypertensive heart disease, myocardial infarct (old)	Left	++	Limited by right-sided angina.	4 mos.	Alive at 5 yrs.		
70A	*Rev. H. K.	60	Coronary heart disease, myocardial infarct (old).	Left	+	Continued as minister until death.	0	1 yr.	Acute coronary insufficiency.	0
71A	Morris L.	70	Coronary and hypertensive heart disease, myocardial infarct (old), angina decubitus, diabetes.	Right.	0	Limited by mild congestive failure.	0	2 yrs.	Congestive failure.	0
75A	Donald McR.	57	Coronary and hypertensive heart disease.	Left.	+++	Limited by painless equivalents of angina pectoris.	0	3 mos.	Coronary thrombosis (autopsy).	0

TABLE II—Continued

NO.	PATIENT	AGE	ÆTIOLOGY	INJECTION	TEMPORARY NEURALGIA	ACTIVITY AFTER INJECTION	RECU- RENCE	SURVIVAL	CAUSE OF DEATH	PAIN AT DEATH
2. Fair Results—10 Cases										
2A	Harry T.	60	Rheumatic, hypertensive, and coronary heart disease, aortic regurgitation with slight congestive failure, coronary thrombosis (old).	Left	+	Resumed light work.	0	7 mos.	Auto coronary insufficiency.	?
3A	*Minnie C.	53	Hypertensive and coronary heart disease.	Left	+	Sedentary.	0	2½ yrs.	Empyema.	
5A	George C.	50	Hypertensive and coronary heart disease, coronary infarct (old).	Left	+	Unable to resume work.	0	4 mos.	Auto coronary insufficiency.	?
7A	*Injection performed by Dr. W. J. Mixer	53	Hypertensive and coronary heart disease.	Right	?	Resumed light work.	0	Alive at 5 mos.		
8A	Injection performed by Dr. W. J. Mixer	68	Hypertensive and coronary heart disease, coronary infarct (old).	Left	?	Mild activity indoors.	0	10 mos.	Auto coronary thrombosis.	?
20A	Patrick S.	63	Coronary and hypertensive heart disease, aortic regurgitation.	Left	+	Resumed practice of law.	0	17 mos.	Congestive failure.	?
31A	*Dr. David B.	53	Coronary heart disease.	Left	+++	Limited by dyspnoea.	0	Alive at 8 mos		
34A	Fred F.	69	Coronary and hypertensive heart disease. Obesity. Prostatism.	Left	+	Moderate, in spite of frequent but much milder attacks.	0	10 yrs.	Coronary thrombosis.	0
36A	Mary S.	37	Rheumatic heart disease with aortic regurgitation, angina decubitus.	Left	0	Mild angina pectoris L. arm.	0	Alive at 3 mos.		

42A	Roscoe W	54	Coronary heart disease, myocardial infarct (old), angina decubitus	Left	++	Inactive for remaining month of life	0	1 mo	Acute coronary in sufficiency	0
44A	Arthur L	52	Coronary heart disease and angina decubitus	Left	++	Sedentary	0	Alive at 9 mos		
47A	Harry K	61	Coronary and hypertensive heart disease previous congestive failure	Left	+	Moderate, but no work	0	3 mos	Acute coronary in sufficiency	Arm (left)
55A	Caroline D	72	Coronary heart disease, myocardial infarct (old)	Left	++	Limited by dyspnea and congestive failure	0	8 mos	Congestive failure (?)	?
57A	*Albert B	60	Coronary heart disease	Bilateral	++	Continued work as chauffeur	0	2½ yrs	Acute coronary in sufficiency	Precordial
72A	Regina C	47	Syphilitic, coronary, and hypertensive heart disease, previous myocardial infarct and angina decubitus	Left	++	Limited by dyspnea and congestive failure	0	Alive at 5 mos		
74A	Sidney L	62	Coronary heart disease with 3 previous myocardial infarcts, severe nocturnal angina	Bilateral	++	Able to do light office work, but considerable angina with reference to low precordium and jaw	2 mos	1½ yrs	Acute coronary in insufficiency	+

3 Failures--6 cases

NO	PATIENT	AGE	ÆTIOLOGY	INJECTION	TENTH- EARY NEURAL- GIA	ACTIVITY AFTER INJECTION	SURVIVAL	CAUSE OF DEATH	PAIN AT DEATH
16A	*Thomas S	57	Coronary heart disease	Left	++	Unchanged	Alive at 6 wks		
17A	Chas McK	53	Syphilitic aortitis with angina decubitus	Left	++	Unchanged	2 mos	?	?
49A	*Matty J	43	Coronary heart disease	Left	++	Unchanged	14 mos	Acute coronary in sufficiency	
50A	*Albert D	56	Coronary and hypertensive heart disease	Left	+	Unchanged	7 mos	Acute coronary in sufficiency	+
60A	*Julia M	66	Coronary and hypertensive heart disease, angina decubitus, diabetes	Left	+	Residual pain in arm	Alive at 11 yrs		
65A	Nathan S	52	Coronary heart disease, chronic lymphatic leukemia	Left	++	Unchanged	4 yrs	Acute anuria and uraemia	0

TABLE II—Continued
4. Unclassified Because of Brief Period of Observation—5 Cases

NO.	PATIENT	AGE	ÆTIOLOGY	INJECTION	TEMPORARY NEURALGIA	IMMEDIATE RESULT	PERIOD OF OBSERVATION	SURVIVAL	CAUSE OF DEATH	PAIN AT DEATH
14A	William T.	54	Coronary disease with recent infarction, congestive failure, and angina decubitus.	Left	+	Excellent	2 wks.	?		
33A	Arthur R.	70	Coronary heart disease, bronchial asthma.	Left	0	Excellent	1 mo.	1 mo.	Asthma and congestive failure.	0
52A	Reginald P.	43	Rheumatic heart disease with aortic and mitral regurgitation and stenosis	Left	?	Excellent	2 wks.	?		
63A	Joseph B.	45	Coronary heart disease, myocardial infarct (recent), and angina decubitus.	Left	0	Excellent		6 mos.	Coronary thrombosis.	+
73A	*Dr. N. L.	51	Angina decubitus (see case history in text).	Left	+++	Excellent	Posterior rhizotomy after 2 mos.	Alive at 6 yrs.		?

5. Post-injection Deaths—6 Cases

NO.	PATIENT	AGE	ÆTIOLOGY	INJECTION	RELIEF OF CARDIAC PAIN	COMPLICATION	SURVIVAL
10A	Injection performed by Dr. W. J. Mixer	51	Coronary disease with recent thrombosis and morphinism.	Left	Complete	Recurrent coronary thrombosis.	3 weeks.
25A	Mrs. R.	85	Coronary disease and exhaustion from angina decubitus.	Right	Complete	Pneumonia.	3 days.
32A	Mrs. Fred D.	50	Syphilitic aortitis and hypertension, angina decubitus.	Left	Complete	Acute coronary insufficiency.	22 hrs.
40A	Hunter C.	35	Rheumatic heart disease with aortic regurgitation.	Left	Complete	Post-injection pleuritic pain probably contributed to ensuing congestive failure.	10 days.
53A	James S.	62	Coronary heart disease with recent myocardial infarction.	Left	Complete	Coronary and cerebral thrombosis.	12 days.
58A	William K.	65	Coronary heart disease, myocardial infarction (old).	Right	Complete	Acute coronary insufficiency.	Died during injection.

cent listed as good results are included those patients who were completely or nearly completely relieved of their pain on the side of injection. The 21.3 per cent with fair results comprise a group in whom intractable angina pectoris was so reduced that the patients could be maintained in a state of relative comfort by routine medical measures without the use of narcotic drugs. If we exclude the 5 patients in the group which are unclassified because of inadequate follow-up or other reasons (all of whom appeared to have good results), 84 per cent of the entire series obtained excellent to fair results, so that even the least satisfactory felt that they were distinctly improved. There were 8 per cent who failed to derive adequate relief and are classified as failures, and 8 per cent died as a direct result of the procedure. Although this mortality rate is nearly equal to that following operation, it is important to bear in mind that this group includes the very worst risk cases with severe coronary disease, threatened decompensation, extreme old age, and other conditions which would have resulted in a prohibitive mortality had any neurosurgical procedure been attempted. This was the situation with Case 48A, who came through a bilateral injection without any disturbance and subsequently succumbed to an operation on his gall bladder. Intercostal neuralgia, while a cause of minor discomfort of several weeks' duration in most cases, was a serious complaint in 10 per cent, in whom it was troublesome over periods of one to three months, but ultimately subsided.

In recent years with improvement in injection technique the proportion of good results has increased steadily, so that the results in the latter third of the series are distinctly the best. The introduction of x-ray control in the placement of the needles barely antedated the war and has therefore been tried on only a single case. This should lead to much greater accuracy in blocking the cardiac rami and be reflected by still more impressive results.

In the group of patients who have had good initial results from chemical block, partial to complete recurrence of pain secondary to recovery of nerve conduction has been observed in 18.7 per cent after periods of from two and one-half months to five years, but, on the other hand, most of the remaining ones are known to have maintained full benefit for periods ranging from one to nine years. It seems remarkable that mere infiltration of alcohol could block the cardiac sensory fibres over such a prolonged period, as we have rarely observed the persistence of a Horner's sign, vasodilatation, or anhidrosis for over a year. Nevertheless, in 4 patients with persistent mild anginal attacks on the uninjected side there has been continued absence of pain on the injected side for periods ranging from two to six and one-half years. In other cases, however, it is likely that the long lasting results were due, at least in part, to spontaneous development of a competent collateral circulation in the coronary vessels.

The following case histories illustrate the various forms of cardiac pain which have been treated, and some of the more outstanding results.

Case 1A. William M., 54. Syphilitic aortitis and aortic regurgitation with angina pectoris.

This patient was the first to be treated by paravertebral alcohol injection. He was a middle-aged carpenter who had had syphilis for many years. Nearly three years prior to admission he had his first attack of angina pectoris, and the pains were soon recurring three to four times a day. Syphilitic heart disease was diagnosed in the cardiac clinic. He was there discovered to have marked hypertrophy and dilatation of the left side of the heart with widening of the aortic arch. There was a very loud aortic diastolic murmur, in addition to a moderate aortic systolic and mitral systolic and a diastolic murmur of the Austin Flint type. His pulse was of the Corrigan type and the blood pressure was 170/35. An electrocardiogram showed inverted T-waves in the first and second leads, with left axis deviation.

The patient was admitted to the hospital, where he was kept in bed on medical treatment for over six weeks. During this period his angina increased in both severity and frequency. The most troublesome feature of his attacks was that they came for the most part at night, so that he became exhausted from lack of sleep. As a result the patient and his physicians finally realized that he would die of exhaustion unless relief could be obtained by surgical means. This led Dr. P. D. White to urge a trial of paravertebral alcohol injection, which had recently been recommended by Swetlow (7).

2/12/27: Diagnostic paravertebral procaine block, T₁-T₈ (left), resulted in freedom from his attacks for thirty-six hours, but not for the long period described by Mandl. 2/21/27: Paravertebral alcohol injection, T₁-T₈ (left). The injection was performed without complication and gave the characteristic chest wall anaesthesia, but without a Horner's sign. He reacted differently from all our other patients in that he noted postoperative attacks of decreasing frequency for two weeks, from which time he had no attack on his left side. He was able to return home and lead a quiet life in comfort.

A year later milder attacks were recurring in his right arm and chest wall. An attempt was made to stop these by a right-sided injection by another surgeon, but this block and another later attempt were unsuccessful. He was then followed in the out-patient clinic and remained altogether free of left-sided pain and without too great discomfort on the right, where the attacks could be relieved by nitroglycerine. Finally in 1933 cardiac failure developed from which he died. No autopsy was performed. The relief of his unbearably severe left-sided angina pectoris had lasted over six years.

Case 29A. Dr. William N., 58. Arteriosclerotic heart disease, coronary infarction, and angina pectoris.

A physician, who had enjoyed excellent health, began to notice precordial pain on exertion at the age of 43. Two years later he had a myocardial infarct, followed by an embolus to his popliteal artery. He was incapacitated for three months, but recovered sufficiently to be able to return to his practice. During the past eleven years he had been fairly active, but had suffered from frequent attacks of angina pectoris. The pain was substernal and radiated to the precordium and left arm. He obtained quick relief from nitroglycerine until four months prior to his admission, but at that time he had a series of unusually severe attacks lasting one to two hours and requiring morphine for relief. Three days before entry one of these attacks lasted four hours. As all medical measures had failed, paravertebral injection was recommended by Dr. P. D. White. The patient's father had died of coronary thrombosis and his mother of arteriosclerosis. His own past history was not pertinent to his present illness. Physical examination was essentially negative except for his cardiovascular system. There were no abnormal pulsations in the veins of his neck, nor any evidence of congestive failure. The blood pressure was 150/90. An x-ray of his heart demonstrated no abnormality except a tortuous aorta. The electrocardiogram showed a normal rhythm, rate 75, left axis deviation, and diphasic T₁.

4/25/33: Left paravertebral procaine-alcohol injection, T₂-T₄. The patient complained of very little discomfort from this procedure. He developed a striking vasodilatation of his left hand and cessation of perspiration, as well as a Horner's sign. The post-operative x-ray showed a slight degree of pneumothorax (probably from penetration of the lung during the

insertion of the needles), but this subsided within a few days. A letter received six weeks later reported that he had again returned to his practice and was totally free from attacks. Furthermore, he had no discomfort in the anaesthetic area in his chest. During the next fourteen months he carried on moderately active work and remained free of left-sided angina pectoris. He had, however, noticed the onset of pain in his right anterior chest. At first this had been a useful warning signal, but lately it had become increasingly severe. He was so pleased with the result of his left-sided injection that he re-entered the hospital on 6/12/34 for a similar procedure on the right. On the day of his admission he had attended his daughter's college graduation exercises and noticed an unusual amount of right-sided pain as he walked to his room. At midnight he was awakened by terrific pain in his right chest, which caused him to go into collapse; there was only a slight sense of oppression on his left side. During the course of this attack his blood pressure fell, respirations became labored, and he died three hours later.

At autopsy there was nothing remarkable outside the heart, which showed diffuse calcification and areas of occlusion and recanalization in both coronary arteries. There was marked scarring of the left ventricle and septum. No recent thrombus could be made out. The only evidence of the old alcohol injection was thickening of the pleura in the region of the second and third thoracic ganglia. Cause of death: Acute coronary insufficiency.

Case 35A. Evelyn C., 26. Rheumatic heart disease with free aortic regurgitation and angina pectoris. (This patient with a group of other patients who developed angina pectoris secondary to valvular disease of rheumatic origin has already been reported by Bland and White (37).)

The patient had rheumatic heart disease with marked cardiac enlargement, free aortic regurgitation, blood pressure of 170/50, mitral stenosis and regurgitation, and angina pectoris decubitus. Severe rheumatic fever and heart disease began at the age of 9 years. A recrudescence of rheumatic fever occurred at the age of 16, requiring hospitalization for twelve months. She subsequently did well and remained free of symptoms except for moderate exertional dyspnoea and palpitation until at the age of 26 she re-entered the hospital with another recrudescence of rheumatic fever. While at rest in bed she began to have severe angina pectoris. Her attacks were characterized by paroxysmal discomfort due both to pain and to associated circulatory phenomena. The sequence of events began with consciousness of forceful regular heart action and a sense of throbbing in the throat, accompanied by an increase in the pulse rate from a resting level of 90 up to 130 or 140 per minute. In one to two minutes an aching precordial pain appeared, rapidly becoming severe and spreading upward in the chest and down the left arm as far as the wrist. Respiratory discomfort and a sense of choking were usually present, as well as profuse sweating and generalized flushing of the skin. A blood pressure determination was not made during an attack. Occasionally dyspnoea and palpitation occurred without pain, but never the reverse. Although precipitated by emotion or exertion, the attacks most frequently occurred without provocation, especially during the night. The severe anginal pain was usually superimposed upon a less intense precordial aching sensation similar to that frequently described by patients during active rheumatic fever. Nitroglycerine gave partial relief, but it was this latter component of the patient's discomfort which remained uninfluenced by the drug and for which morphia was frequently required.

5/31/34: Paravertebral alcohol injection, T₁-T₄ (left). There resulted a well-marked Horner's syndrome, a transient partial anaesthesia over the left chest anteriorly, and a variable paraesthesia over the left upper back and down the inner aspect of the left arm. This was followed by complete relief from the anginal pain during frequent attacks, the presence of which was made known by a tightening sensation in the throat and a persistence of the accompanying palpitation, respiratory discomfort, and generalized flushing of the skin. However, another important element in addition to the pain had been dispelled, namely, the fear of an impending attack. It is of considerable interest that the precordial ache, which previously had not responded to nitroglycerine, persisted off and on in a modi-

fied form, but on the whole was less severe and less frequent. This component appeared to be directly related to the active rheumatic disease and subsequently entirely disappeared. The patient was seen at frequent intervals and was examined in June, 1936, two years after the injection. She was in good condition and was free from clinical and laboratory evidence of active rheumatic infection. There remained a slight residual Horner's syndrome and a vague sense of numbness to touch over the precordial area, with slight paraesthesia along the inner aspect of the left upper arm. She led a quiet life and was able to do light household work. About once a week she had to pause for a few minutes because of tightening in the throat and thumping of her heart, but this was now always related to unusual exertion or excitement.

Nearly four years after the injection the patient developed subacute bacterial endocarditis, from which she died. She remained free of her old anginal attacks throughout.

Case 39A. James I., 63. Arteriosclerotic heart disease, previous coronary infarction, and angina pectoris.

This patient was seen in the University Hospital, Charlottesville, Virginia, in consultation with Drs. A. D. Hart and J. E. Wood. His angina pectoris dated back over an eight-year period, but he was able to get along quite comfortably on medical treatment until the spring of 1935. At that time he had a fairly severe attack of coronary thrombosis, from which he made a slow convalescence, but thereafter his anginal attacks became more severe, increasing in frequency up to thirty or forty a day. The attacks radiated to both arms and were especially severe at night. He had been in the hospital for over a month while unsuccessful attempts were made to give him rest at night with oxygen inhalations and opiates. The patient was obese. He had moderate peripheral arteriosclerosis and a blood pressure of 170/95. The cardiac dulness could not be determined with great accuracy, but it was thought that the heart was enlarged. Its sounds were of fair quality and there were no murmurs. As it was believed that the patient could not long survive the exhaustion brought on by his loss of sleep from pain, it was hoped that bilateral alcohol injection might give him much needed relief.

10/8/35: Paravertebral alcohol injection, T₁-T₄ (left). 10/9/35: Paravertebral alcohol injection, T₁-T₄ (right). The patient came through both injections with a minimum of discomfort and proceeded to recover in a way that exceeded all our hopes. He never had another attack of cardiac pain, but continued to have a satisfactory warning signal, which consisted of a sense of oppression in his suprasternal notch. With adequate sleep he was soon able to leave the hospital, and in a remarkably short time to resume mild activities in his store. When seen six months later he was at work and free of pain. Unfortunately, ten months after injection he had a flare-up of an old subacute cholecystitis, for which his medical advisers were not consulted. Operation, which was performed at another hospital, resulted in an early death from congestive failure.

In commenting on this case his physician, Dr. J. Edwin Wood, wrote as follows: "I would not hesitate to say that Mr. James I.'s relief following paravertebral block was as nearly complete as anything I have ever seen. His relief was so complete, in fact, that he was back at work in a relatively short time and experienced no discomfort while at work. He told me that on occasions with exceptional exertion, i.e., exceptional for him, he could feel perhaps a little pressure sensation in his chest which he rather felt was a warning and was pleased that it was there. . . . Mr. I.'s life was unquestionably prolonged by the paravertebral block and I feel that if he had not insisted on the gall bladder operation he would have had a number of months and perhaps even years ahead of him. It was truly amazing to see him back at work in the grocery store after having followed him for some weeks in a perfectly miserable state."

The next two case histories illustrate equally dramatic recoveries from states of totally incapacitating angina pectoris with recovery of normal activity and long survival.

Case 51A Mrs Amelia F, 66 Hypertensive heart disease and angina pectoris

This woman, referred by Dr Robert L Levy, had suffered from attacks of left sided angina pectoris for ten years. These became much worse following an infarction in 1936, so that she was restricted to her room and required morphine for relief of the pains, which failed to respond to nitroglycerine or codeine. These attacks often occurred in bed and she was losing sleep and weight. The electrocardiogram had shown progressive changes with inversion of T₁ and T₂ and upright T₄. There were left ventricular preponderance and a slow rate from 40 to 60.

4/17/37 Left paravertebral injection, T₁ to T₄, was performed in her room. She was able to be up on the following day and had a minimal degree of neuralgia during the next few weeks. With the disappearance of her anginal attacks she no longer suffered from insomnia and her weight and cardiac reserve were regained rapidly.

Nine years later Dr Levy reports "She is now 75 years old. In her case the injection of alcohol remade her entire life. She has been completely free from cardiac discomfort for several years. She is very active and is a director of the Home for Old People. She goes out to dinner two or three times a week and plays bridge frequently. She goes to the theatre and the movies. She is happy and in remarkably good health for a woman of her age. The last electrocardiogram was made on April 14, 1943. At that time there was a well marked sinus arrhythmia and left axis deviation. There were no changes indicating myocardial damage. The blood pressure was 151/86. It is my opinion that the injection prolonged this patient's life, although this point would be difficult to prove."

The improvement in the second electrocardiogram reported by Dr Levy is noteworthy.

Case 61A Reginald F, 46 Arteriosclerotic heart disease with coronary occlusion and angina pectoris

This retired army officer was referred to us by Dr H M Marvin of New Haven. Since the last war he had had "soldier's heart" with easy fatigue, dyspnoea and palpitation. Seven months before he had a sudden attack of precordial pain with radiation to both arms which required morphine for relief. Three months prior to admission he had another severe attack of precordial pain. He then began to suffer frequent and intense attacks of bilateral angina pectoris. As this patient was a high strung, strenuous man, he reacted poorly to inactivity and could not be controlled by medication. His heart was not enlarged, sounds were of fair quality, no murmurs, rate and rhythm were normal. Electrocardiogram showed inverted T waves in leads 2 and 3, with a normal chest lead.

10/27/38 and 10/29/38 Left and right sided paravertebral injection of alcohol. These were followed by more than the usual degree of neuralgia, as had been anticipated from his nervous make up, but the thoracic discomfort subsided in a little over a month. He then returned to Bermuda. Six months later he reported that he was "working and exercising almost normally." Two years after injection he was able to pass his army medical examination. On nervous or physical strain he experienced a warning signal which consisted of "slight congestion on the sides of the throat or aching in the left arm like rheumatism." In January, 1941, his local doctor reported him to be "absolutely well in all respects." At the onset of the war he tried to return to active service and would have been reinstated in his commission had it not been for the past history of angina pectoris. In June, 1945, he was reported as lost at sea in a small sailboat off Bermuda.

A final case history is of interest from another angle, as it illustrates a small but important sub group of cases of angina pectoris in which pain is experienced during attacks of paroxysmal tachycardia or auricular fibrillation. In these there is suggestive evidence that sympathetic denervation is capable not only of interrupting the pain, but of eliminating the cardiopressor impulses which give rise to the attacks (27).

Case 43A. Abigail W., 69. Coronary heart disease with paroxysmal auricular fibrillation and angina pectoris.

Attacks of rapid irregular action had been recurring for eleven years prior to this woman's admission. Two years ago these had become more frequent and accompanied by precordial pain radiating to both arms. The electrocardiogram showed evidence of coronary disease. On entry to the hospital in 1936 these attacks were occurring two to three times a week and lasting several hours. Therapy with quinidine and digitalis was not effective. Examination showed left-sided cardiac enlargement, blood pressure 120/70, and thickened and tortuous peripheral arteries. At the times of her attacks the heart rate rose to 140 with grossly irregular action. There was no evidence of congestive failure. The electrocardiogram, taken at a period of normal heart rate, showed a flat T_1 , sagging ST_2 and ST_3 and diphasic T_4 with initial phase inverted. At the time of one of these painful episodes there was auricular fibrillation with a ventricular rate of 120 and a diphasic ST_2 and ST_3 .

2/8/36: Paravertebral alcohol injection T_1 - T_4 (right). 2/20/36: Similar injection on left. Although the attacks of paroxysmal cardiac irregularity continued after the first injection, they were painless on the injected side. After the left side was blocked they ceased altogether for six months, with the exception of a single attack, and did not again become really troublesome for twenty-one months.

At her second admission in December, 1937, they were again occurring with equal intensity and pain. Alcohol injection was therefore repeated bilaterally and again stopped the episodes of tachycardia, but this time only for a month.

This patient had very little discomfort from the injections, and it was evident that blocking the cardiac nerves was capable of stopping the pressor discharge which initiated the arrhythmia and also of blocking the afferent fibres which carried her pain. Evidence for this is the elimination of her right-sided pain after the first injection, when her attacks were still in progress. To-day we would recommend a bilateral upper thoracic sympathetic ganglionectomy, after demonstrating that nerve block with procaine would stop an attack. Another instance of stopping attacks of paroxysmal tachycardia by sympathetic denervation of the heart has been described by one of us (27), together with a review of the literature on this subject.

2. Upper Thoracic Ganglionectomy

Study of the statistics summarized in Table III shows that resection limited to the upper three thoracic ganglia is nearly certain to afford complete relief of pain on the side of operation. Whether posterior fixation of the cardiac rami may require additional resection of the fourth thoracic ganglion in rare instances remains, as yet, uncertain.* Olivecrona (22) in Sweden, following the technique recommended by one of us, has now performed this operation upon 73 patients. He reports excellent results and a mortality rate of only 6.8 per cent. In our smaller series of 8 cases there has been complete relief of pain in the precordium and arm on the denervated side. In one of our earlier patients, Case 3B, there was late recurrence of pain referred to the arm and possibly some return in the left precordial area. A very slight return of crushing sensation in the right an-

* A number of years ago Raney (38) advocated simple resection of the sympathetic rami which are given off by the upper thoracic sympathetic ganglia without resection of the latter structures. In view of the extraordinary capacity for regeneration which has been observed (27) in the surgical treatment of Raynaud's disease, we believe that this suggestion was a serious mistake. Once these ganglia have been exposed it is advisable to remove as great an extent of both the main paravertebral chain and its rami as possible.

TABLE III
*Relief of Pain in Severe Angina Pectoris—By Upper Thoracic Sympathetic Ganglionectomy—8 Cases**

NO	PATIENT	AGE	ÆTIOLOGY	OPERATION	RELIEF	ACTIVITY AFTER OPERATION	SURVIVAL	CAUSE OF DEATH	PAIN AT DEATH
1B	Giuseppe C	29	Rheumatic heart disease with aortic and mitral lesions. Severe long lasting attacks of angina pectoris, especially at night	T ₁ and T ₂ (L)	Good	Overactive in business despite pain less warning signal. Took no care of himself	7½ months	Cardiac decompensation	Right side only
2B	Charles A	29	Syphilitic aortitis and aortic regurgitation, angina pectoris with radiation to both arms	T ₂ -T ₃ (L)	Good	Survival period too short to evaluate	12 days	Post-mortem. Lucetic occlusion coronary orifices, total on R, 50% only on L	Right side only
3B	Mrs A B	60	Arteriosclerotic heart disease with coronary involvement	T ₁ -T ₂ (L)	Good	Led active life but suffered partial recurrence after first year	5 years	Probable coronary thrombosis	?
4B	Nathan O	62	Arteriosclerotic heart disease with coronary infarction	T ₁ -T ₂ (L)	Good	Survival period too short to evaluate	1 month	Empyema secondary to post-op pneumonia	0
5B	Mrs E P	53	Arteriosclerotic and hypertensive heart disease	T ₂ -T ₃ (L)	Good	Moderately active life with mild attacks in lower jaw at 15 mos			
6B	Dr I W	44	Hypertensive heart disease with severe bilateral angina pectoris and drug addiction	Inferior Cerv T ₃ (L) T ₁ -T ₂ (R) T ₂ -T ₃ (L)	Fair Good Good	Continues practice of medicine at 5 yrs. Mild residual pain in lower jaw			
7B	Charles C	47	Arteriosclerotic heart disease with angina decubitus and severe right-sided A P	T ₁ -T ₂ (R)	Good	Restricted activity at 4 yrs			
8B	James T	44	Arteriosclerotic heart disease with coronary involvement	T ₁ -T ₂ (L)	Good	Active work as tax investigator at 3 yrs. Little need for nitroglycerine			

*Drs W J Mixer and A W Allen helped with the operations in the first four patients

terior chest has also been reported at six years by another patient (see protocol of Case 6B below). The fact that this has recurred in association with a definite recovery of sweating in the face and arm is evidence of nerve regeneration. There has been no complication of this sort in any other patient. There was one death which, although it occurred a month after operation, must nevertheless be ascribed to surgery. This patient developed a staphylococcus aureus wound infection and pneumonia, from which he apparently made a good recovery and was discharged after twenty-seven days. A week later, however, he died of a painless coronary occlusion. Postmortem examination showed an encapsulated empyema in the paravertebral gutter. There were sclerotic plaques which had caused extensive occlusion of both the right and left coronary arteries, with areas of old and recent infarction. Postoperative infection was undoubtedly the precipitating factor in his death. Another such complication should now be preventable by chemotherapy.

The following case histories illustrate a number of interesting features in regard to the extent of neurectomy necessary to ensure complete interruption of anginal pain and the striking degree of improvement in the patient's physical and psychic condition which follows relief.

The first shows the limited unilateral effect of a left-sided denervation by the fact that in the eventual fatal coronary insufficiency intense pain involved the right arm and right anterior chest, but never crossed the midline.

Case 1B. Giuseppe G., 20. Rheumatic heart disease, mitral stenosis and regurgitation; also aortic stenosis and regurgitation with angina pectoris. This young man first entered the hospital with rheumatic fever in 1925. At that time he already had signs of severe cardiac involvement with free aortic regurgitation. In 1928 he experienced precordial pain on drinking cold water. Since then the attacks had remained localized to the left precordium, but increased in number and severity. They were particularly troublesome at night, averaging four to six attacks, and lasted as long as an hour.

Dr. P. D. White's examination revealed a pale, thin young man with bounding arterial pulsations in his neck, a thrill over the great vessels, and both systolic and diastolic aortic murmurs. There was no evidence of cardiac failure. The heart was moderately enlarged. Electrocardiogram showed a diphasic T₂ and left axis deviation.

1/28/29: Diagnostic procaine block of first and second thoracic ganglia, followed by relief for twenty-four hours.

2/5/29: Resection of central end of second rib with first and second thoracic sympathetic ganglia on left side by Drs. W. J. Mixter and J. C. White. The patient made a smooth convalescence. His left-sided anginal attacks were permanently relieved, but he continued to have mild bouts of pain in his right chest, which served as a warning signal. He left the hospital and continued to work as an insurance agent and to lead a fairly active life for the next eight months. He was then forced to re-enter the hospital on account of progressive dyspnoea. On the third day he developed fatal coronary insufficiency with severe pain, which was observed from its onset. The remarkable feature of this attack was the distribution of his pain, which was confined entirely to the right side of the anterior chest and stopped exactly at the midline. Postmortem examination could not be obtained.

Another patient illustrates the difficulties which may be encountered by peculiar reference of pain to the head and jaw. The typical anginal pain in

the chest and arm was relieved by resection of the inferior cervical and upper thoracic ganglia, but the unusual radiation to the upper cervical dermatomes was not interrupted either by resection of the superior cervical ganglion or by subsequent division of the superficial branches of the cervical plexus.

Case 5B. Mrs. Elizabeth P., 58. Arteriosclerotic and hypertensive heart disease with angina pectoris.

Mrs. P. had a striking hereditary background of degenerative vascular disease. She herself had had high grade hypertension for the past twelve years without complications. For the past three years she had suffered from angina pectoris. The attacks, which were entirely localized to the left side, involved the precordium and arm in the usual manner; in addition pain radiated to the forehead, where it was felt behind the eye, to the upper and lower jaws, and also to the neck and posterior scalp. Before entering this hospital she had been treated by Dr. H. M. Marvin by rest in bed for several months without relief. Dr. Marvin then referred her to us for operation.

The patient was an intelligent and most coöperative woman of slender build. Physical examination showed tortuous radial arteries and a blood pressure of 270/130. There were no signs of congestive failure. By x-ray the left ventricle was slightly enlarged and the aortic arch tortuous. The electrocardiogram showed "coronary" T-waves.

5/10/39: Resection of the left inferior cervical, first and second thoracic sympathetic ganglia. The resection, made through the supraclavicular approach, was followed by an uneventful convalescence. The attacks of precordial and arm pain were relieved, but she continued to feel pain radiating to her neck, scalp, and face.

7/5/39: Resection of the left superior cervical ganglion. Following this operation, which divided some of the branches of the superficial cervical plexus, the skin of her neck was at first anaesthetic. During this period she had no real pain, but noticed clutching sensations in her throat and some discomfort in the left side of her face on over-exertion. As cutaneous sensation in her neck recovered, pain again recurred in this area and became particularly troublesome in the left occipital area. Because she had experienced relief during the period of cutaneous anaesthesia, it seemed logical to try the effect of permanent denervation of the area to which this unusual pain was referred. This was accomplished by subsequent resection in two stages of segments of the great occipital and other branches of the superficial cervical plexus, but was followed by only a transitory period of relief. A year and a half later the patient wrote that the first operation "removed completely all pain from the lower chest, over the heart, and in the arm. . . . I can truthfully say that in spite of the fact that the last two operations have numbed superficial areas, they have not prevented the recurrence of the deep pains" in the base of the neck, chin, and posterior scalp.

The exact mechanism of this high reference of pain is obscure (see below).

A sixth case in this series is particularly instructive because it shows the unimportance of removing the lower cervical ganglia, but the vital need for carrying the resection caudally to include the third thoracic ganglion. While this is not always necessary, as demonstrated by Cases 1B and 5B, its neglect will certainly result in persistent low precordial pain in a fair number of cases.

Case 6B. Dr. E. W., 44. Hypertensive heart disease with bilateral angina pectoris and morphine addiction.

This woman physician came from a family predisposed to cardiovascular disease. She suffered a coronary occlusion in 1939 and thereafter developed frequent attacks of crushing pain in the precordium with radiation to both arms. Nitroglycerine would abort but failed to relieve the attacks, for which she had taken opiates and developed a definite addiction to the drug. Examination revealed a nervous, high-strung woman who was suffering

severely from insomnia and pain, so that she had been taking increasing doses of dilaudid and nembutal over the last two months. Her heart was normal as to size and sounds. Blood pressure was 160/110. The electrocardiogram showed evidence of slight left ventricular strain (moderate left axis deviation). It was believed that she would have to be given complete relief in order to lessen her nervousness and eliminate her drug addiction, so for this reason and with her agreement a bilateral sympathectomy in two stages was planned.

10/22/41: Supraclavicular approach and resection of middle and inferior cervical with upper two thoracic sympathetic ganglia on left side. After this operation there was relief of all pain on the left side except for a slight residual distress under her breast, but with the continued right-sided attacks her convalescence was complicated by psychic changes secondary to her drug addiction.

12/15/41: Posterior approach through second rib with removal of upper three thoracic ganglia on right side. Convalescence after the second operation in the absence of painful anginal attacks was more satisfactory. Her warning signal now consisted of episodes of painless dyspnoea. These attacks, which were controlled by nitroglycerine, diminished in number from a preoperative frequency of up to 40 a day to between 6 and 8. After a period of two months' psychiatric rehabilitation she returned to her home and practice. She was then able to continue office work for a period of seven months, when she had what appeared to be another coronary occlusion. Thereafter she again began to complain of pain low in the left precordium, owing to our failure to resect the third thoracic ganglion. She was readmitted at her own request to complete the denervation of the left side of her heart.

4/6/43: Resection of third and fourth thoracic ganglia on left through third rib approach. An additional 4 cm. of the paravertebral chain was removed, including the dural clips applied to the distal stump at the previous intervention. Convalescence was uneventful and nursing care far less difficult than at her former admission. Fifteen months later she reported that she had no precordial pain, but infrequent warning signals on over-exertion. These consisted of "a slight, but really indescribable sensation in the chest where the old bouts of pain used to be" and some mild but definite pain referred to her neck and lower jaw. "I am more comfortable than I have been in six years. Walking is out, but I can drive without difficulty. . . . Following my doctor's advice, I only practise about three hours daily. . . . Last electrocardiogram is not appreciably changed."

Now, five years after her initial operation, she reports that she has never had any precordial or arm pain, but that if she over-exerts she is "brought up shortly by pain in the neck and jaw. This as a rule is quite bearable and passes in a couple of minutes. . . . I put in an average of six very busy hours daily in medical work. . . . I have noticed during the last few months that when I have an occasional severe attack there is a slight crushing in the chest on the right side. Shortly after noticing this for the first time I observed that such attacks were accompanied with some perspiration of the upper half of the right side of the face and slight moisture of the right palm. . . . In 1941 I felt that I had reached the extreme limit of my endurance and now I am again living a full life."

The next patient in this series had been partially incapacitated for many years by right-sided angina pectoris and for three months by angina decubitus. The results of a purely right-sided denervation in this unusual distribution of anginal pain were most satisfactory.

Case 7B. Charles C., 47. Arteriosclerotic heart disease with angina decubitus (right-sided pain).

This veteran of the first world war was seen at the U. S. Naval Hospital at Chelsea at the request of Dr. James Faulkner. He had been incapacitated for work by angina pectoris for thirteen years and complained of deep-seated pain on exertion in his right anterior chest with radiation to his right arm and neck below his ear. For six years he had suffered,

periods of angina decubitus each winter. For the last few months he had been forced to remain in bed and was frequently awakened from sleep. As nitroglycerine was no longer effective he was requiring morphine in increasing dosages. Except for the fact that he was a steady drinker, his past history was uneventful, but his father had died at 51 of "heart disease." There was no history of syphilis and his Kahn reaction was normal. General physical examination was not remarkable. Blood pressure 130/94. The heart was not enlarged, but the sounds were indistinct and there was a late systolic murmur at the apex. The electrocardiogram showed T_2 upright but low, T_3 inverted, and a slight delay in A-V conduction. When an anoxia test was performed with 10 per cent oxygen and 90 per cent nitrogen, substernal pain developed in six minutes with depression of the S-T interval and a diphase T_4 .

4/13/42: Right-sided resection of the lower portion of the inferior cervical and upper three thoracic ganglia through a posterior second rib approach. Convalescence after this operation was quite uneventful. Three weeks later on repeating the anoxia test there was no longer any pain on the right side or any change in the electrocardiogram. The patient noticed mild discomfort only on the left side of his chest. A year later in answer to a follow-up letter he stated that he had not had pain on his right side at any time. "I get a warning, which affects me with a constricting sensation at the base of the throat and a cramping sensation high and to the left of the breast bone. These attacks do not last, as they did before, and usually disappear after a short period of relaxation and a nitroglycerine tablet." A recent letter four years after operation reports continued freedom from pain, although his activity is much restricted.

The final case history in this series is of interest in view of the fact that this patient has taken no precautions as to his activities, to his eating, or to gain in weight since his operation over two years ago.

Case 8B. James T., 44. Arteriosclerotic heart disease with recent coronary thrombosis.

This patient began to notice easy dyspnea with a sense of tightness and compression in the precordium on mild exertion for a year prior to his admission to the U. S. Naval Hospital at Chelsea. Three weeks before he had been seized by a severe long-lasting attack of left-sided pain, for which he was given morphine and kept in bed for ten days. On admission any movement precipitated pain in the precordium and left arm. The patient was an obese, flabby veteran. Dr. James Faulkner, who saw him on the medical service, found his blood pressure to be 150/80, the heart not grossly enlarged, and free from overt disease. The electrocardiogram showed T_1 , T_2 and T_4 to be diphase with a low origin, T_3 upright. Kahn reaction negative. After two months he had failed to obtain adequate relief by careful medical treatment.

6/3/43: Posterior approach through second rib with resection of upper three thoracic ganglia on left side. Recovery was uneventful and the patient was discharged free of pain twelve days later.

It has been possible to get this man back for a recent follow-up examination. Three years after his operation he continues active work as a tax investigator. Although he has had no pain, he has the usual painless equivalent once or twice a day, which is relieved by nitroglycerine. This represents a great reduction in the number of attacks with a remarkable increase in work tolerance. His electrocardiogram has not improved. In spite of an adequate warning signal he has not limited his activities in any way and he continues to eat excessively and is markedly overweight.

Of the last 4 patients submitted to thoracic sympathetic ganglionectomy since 1939 all remain free of their former severe pain at intervals of ten months to six years, and 3 have been able to return to work or to lead moderately active lives. As 3 of these patients live at a distance it has been impossible for them

to report for an examination. On the whole the results in the recent members of this series have been so satisfactory that we are planning the use of thoracic sympathetic ganglionectomy as the preferable surgical procedure in all but the worst risk cases of angina pectoris. It is evident that every precaution must be taken to prevent nerve regeneration and that the operation does not eliminate occasional slight residual pain referred to the jaw. Although the mechanism of this is still obscure, it does not appear to be a serious problem.

3. Posterior Rhizotomy

This operation, as stated above, requires a more extensive removal of bone and a longer period under anaesthesia than simple resection of the ganglia. It should therefore be reserved for the best risk cases and especially for those with bilateral pain. Olivecrona (22), who has given it a trial, believes that ganglionectomy is a safer and more satisfactory operation. It must necessarily remain the procedure of choice for the small group of modern neurosurgeons who are not experienced in the technique of sympathectomy. Rhizotomy is also a valuable recourse in those rare cases where paravertebral alcohol injection

TABLE IV

Relief of Pain in Severe Angina Pectoris—by Posterior Rhizotomy T₁-T₄ or T₅

SURGEON	CASES	DEATHS	RESULTS
L. Davis (Chicago)	1	0	Complete relief at 4 yrs.
W. V. Cone (Montreal)	1	0	Complete relief
F. C. Grant (Philadelphia)	5	1	Residual subclavian pain in 1
H. Haven (Seattle)	6	1	Complete relief at 4 to 10 yrs.
W. G. Crutchfield (Charlottesville)	6	0	Complete relief
B. S. Ray (New York)	11	1	Complete relief

has failed to afford a satisfactory degree of relief and has been followed by a disagreeable neuralgia (see protocol of Case 73A below). We have had no personal experience with this method. The statistics of others are summarized in Table IV. These results demonstrate the effectiveness of the method, as only a single patient had any residual pain. The postoperative mortality has not been high.

The following case history is of particular interest, as it illustrates the benefit of freeing a patient of crippling pain from the point of view of his own peace of mind and restoration to active work over a period nearly six years. We had felt that this doctor was a good risk for thoracic ganglionectomy and had therefore recommended this procedure. Against our better judgment, but at his own request, a paravertebral injection of alcohol was carried out. Cardiac nerve block with alcohol relieved his left-sided pain, but was marred by an unusually severe intercostal neuralgia. Subsequent bilateral posterior root section performed by Dr. Bronson Ray in New York cleared up his neuralgia and put an end to the less severe pain on his right side as well. It is of interest to observe this patient, as has been the case with some of those treated by thoracic ganglionectomy, has developed slight residual pain in the jaw. We are

including this interesting case history with the patient's and Dr. Ray's kind permission.

Case 73A. Dr. L., 51. Arteriosclerotic heart disease with coronary occlusion.

This prominent physician was referred to us by Dr. David Barr of New York. He was a man of outstanding physical and mental energy who had developed mild substernal oppression in 1929. In spite of this he had continued under-water swimming, skiing, and mountain climbing, as well as carrying on a strenuous practice of medicine. In the spring of 1941 he suffered a mild coronary infarction. From then on he did not slacken his work, but took from 8 to 20 nitroglycerine tablets a day with large doses of aminophyllin, and suffered pain on less exertion. On arrival in Boston he was exhausted and having up to eight attacks by day and five by night, associated with dreams that he was surrounded by Germans or about to miss a boat and having to run for it. He experienced pain on both sides of the precordium, and with occasional radiation to the right as well as the left arm. Physical examination showed a stocky and well-muscled man of middle age without cardiac enlargement or murmur. There was no dyspnoea or other evidence of cardiac failure and his electrocardiogram gave no evidence of recent myocardial infarction. There was a suggestion of calcification in his aortic arch. Blood pressure was recorded at 130/80. The Wasserman reaction was negative, and x-ray revealed no evidence of gall bladder disease, but he had a small hiatus hernia. It was the opinion of the medical department that this was not the cause of his pain.

Because of his good general condition surgical denervation of the heart was recommended in preference to injection of alcohol, but the patient elected the latter. Paravertebral injection of procaine followed by alcohol was carried out on the left side on 3/10/42. The early results were excellent, as the patient had little discomfort and was relieved of all his anginal pain, but three weeks later, while recuperating at the New York Hospital, he developed a severe neuralgia in the left chest. With this he noticed a return of anginal pain on the uninjected right side. Two months later this was subsiding when Dr. Bronson Ray carried out a bilateral section of the upper four thoracic posterior spinal roots. After this all vestiges of intercostal irritation disappeared, as well as his residual right-sided angina. He continued to observe an adequate warning signal on over-exertion, which consisted of a sense of painless constriction under his upper sternum. He then returned to his home and to his medical work.

The excellent all around result which followed radical surgery continued after four years. In a letter written in June, 1946, he states that he has never at any time experienced pain in his chest. There is only a mild sense of constriction there and also a sense of actual pain referred to his jaw. Unusual reference of pain to this region has been commented on above. It does not disappear after upper cervical sympathectomy and is probably transmitted by the vagus nerve. Fortunately it always goes away with nitroglycerine and, as it prevents over-activity, he feels that it has a definite beneficial value. His electrocardiogram at present shows a low T-wave in the first lead and the presence of ventricular premature beats. He states that he leaves his house for the hospital at eight in the morning and usually gets back around six, with an occasional afternoon off. "At present I am on the Governor's Hospital Study Commission and the Statchood Committee. I have just finished as president of the County Medical Society and am involved with the . . . dentists on a study of dental decay in relation to diet. Of course, in a business and professional way I am interested in a number of other things, but they are part of work."

V. DISCUSSION

In summarizing experiences with the neurosurgical treatment of medically intractable cardiac pain, we wish to stress that resection of the upper thoracic ganglia or section of the corresponding spinal sensory roots are preferable to

* Since the preparation of this manuscript another follow-up letter written in January 1948 states that he is still free of pain and continues an active practice of medicine.

paravertebral injection of alcohol, provided the patient can tolerate a major operative procedure. These two operations do not cause neuralgia, and abolition of cardiac pain is reasonably certain and permanent. We have indicated in Table II the patients in whom we would now consider ganglionectomy the preferable procedure. This leaves over half of the worst sufferers in whom the risk of operation would have been too serious to contemplate and for whom paravertebral block would still have been the procedure of choice.

From the data presented above we conclude that the nervous connections which carry cardiac pain referred to the precordium and arm are now established. Reference of residual pain to the neck and jaw, which occurred in a few cases, still remains an unsolved problem. It may persist after upper thoracic ganglionectomy (Cases 5B and 6B) or after posterior rhizotomy (Case 73A), but fortunately has never been severe. Resection of the entire length of the cervical sympathetic trunk in Case 5B failed to relieve it. Olivecrona (22), who has encountered similar cases, states that pain referred to the lower jaw can be relieved by injecting the mandibular nerve. The most likely remaining pathway of transmission from the heart to the brain is over the vagus nerves. The sensation in the jaw may be produced by a reflex mechanism similar to that described by Davis and Pollock (39) in their paper on pain from stimulation of the superior cervical sympathetic ganglion.

Provided the immediate complications of acute myocardial infarction or congestive failure are successfully avoided, it is fair to conclude that only good results have followed surgical intervention. Mackenzie's (2) fear that removal of the warning signal of pain would permit the individual to damage his heart by over-exertion has not been substantiated, because the sense of painless constriction and dyspnoea have been effective deterrents. Danielopolu (40) formerly argued that removal of the stellate ganglion was fraught with serious danger to the heart, but evidence for this has also been lacking. Indeed, relief from pain and mental anxiety are most effective therapeutic agents. This has been particularly apparent in Cases 6B, 8B, 1A, 35A, 39A, 51A, 61A, and 73A, all patients with totally incapacitating angina decubitus who were requiring large doses of morphine for relief. The hopeless mental outlook, with drug addiction either threatening or already present, and exhaustion from lack of sleep appeared to limit the period of life expectancy to but a few months. These patients, with the exception of Case 39A, who died at ten months after injection following an ill-advised operation on his gall bladder, have survived for periods of from three to nine years. After nine, four, and six years respectively Cases 51A, 73A, and 8B still continue to lead active and most useful lives. The present examination of the heart and the electrocardiograms in two of these patients now show relatively little evidence of coronary disease.

Tables II and III include the data necessary to evaluate the eventual outcome in those patients who obtained lasting complete remission of cardiac pain. From these statistics alone it cannot be concluded that life is definitely prolonged, except in the group described above where angina decubitus and exhaustion from constant fear, loss of sleep, and morphine addiction seemed to be

rapidly pushing the patient to a fatal termination of his disease. Nevertheless, the freedom from suffering and, furthermore, the ultimate termination of life from painless attacks of coronary thrombosis are most effective arguments for the wider use of surgery in this disease.

In conclusion, there is a small but important sub-group of patients with angina pectoris and complicating paroxysmal auricular tachycardia or fibrillation. Occurrence of severe pain throughout the period of rapid heart action is the rule. This may last from a few minutes to many hours, and the tachycardia is often resistant to quinidine and other medical measures. This combination is not only distressing, but also poses a serious threat to the patient's life with each prolonged attack. Sympathectomy under these circumstances offers almost complete assurance of relief of the pain. Of equal importance is the likelihood of a significant lessening (or actual abolition) of the paroxysms of tachycardia. In this connection it is of interest that Jonnesco's original case (3) probably had this combination. Our Case 43A (see protocol above) illustrates this situation well. Over a six months' period of effective block after paravertebral injection of alcohol she was completely relieved, both of her precordial crushing pain and of disturbing paroxysmal attacks of auricular fibrillation. A second paravertebral injection of alcohol, as is the rule, was less effective, lasting only one month. This patient was not too poor a risk for surgery and it is unfortunate that we did not carry out a bilateral resection of the upper thoracic sympathetic chains.

Remarks Concerning Other Surgical Methods for Relieving Angina Pectoris

Methods described above for the relief of angina pectoris are all based on direct interruption of nerve fibres which carry pain from the heart. Two other interpretations have been made of the beneficial effect of sympathectomy (Leriche (41)). These theories ascribe this to (a) destruction of vasomotor nerves with prevention of constrictor spasm of the coronary arteries, (b) paralysis of sympathetic motor fibres with interruption of cardiopressor reflexes.

As regards the first possibility, a significant degree of coronary vasodilatation following any form of sympathectomy is most unlikely, and after posterior root section there can be no vasomotor effect whatever. Nevertheless, the late results in patients who have had the latter operation are just as good as after sympathectomy, in which the efferent coronary vasomotor fibres are interrupted as well as the sensory afferents. Physiological evidence concerning the action of cardiac sympathectomy on coronary circulation is too conflicting to justify any neurosurgical attempt to increase the blood supply of the myocardium by this procedure. Furthermore, even if it were feasible it would not be a practical procedure, as in the great majority of individuals with angina pectoris the resilience of these arteries has been lost.

Concerning the interruption of motor impulses which drive the heart to exceed its limited capacity for work, the experiments of White, Garrey, and Atkins (19) showed that to accomplish this it is necessary to perform extensive upper thoracic ganglionectomy on both sides. It is also necessary to eliminate

the secretion of the adrenal medullas, because Cannon, Lewis, and Britton (42) have found that the denervated heart is accelerated by minute quantities of adrenine. If it were practical to do this, the heart would be seriously crippled thereby. Our clinical observations have shown that all the sympathetic cardiac nerves can be interrupted and still permit the heart an adequate accelerator response through reduction in vagal tone and the chemical mediation of adrenine and sympathin.

Before leaving the subject of cardiac denervation, the recent modification in operative procedure recommended by Fauteux (26) deserves comment. This writer has proposed resection of the cardiac nerves adjacent to the coronary arteries and ligation of the coronary sinus. This operation requires a wide exposure of the heart. Periarterial dissection of the coronary nerves in the beating heart is at best a difficult procedure, and a considerable length must be resected to prevent their regeneration. In addition, there is little evidence that vein ligation will result in an increased collateral blood flow, especially in the presence of arteriosclerotic vessels. In fact, Beck and Mako (43), after an experimental investigation of ligation of the coronary vein, concluded that its beneficial effects were probably not great enough to justify its application to patients. Although Fauteux reports only 1 fatality in a series of 5 cases, his series is not extensive enough to show that the actual risk may not be far greater* and his period of follow-up is not long enough to prove that cardiac pain will not recur as the nerves regenerate.

While it is our belief that sensory denervation of the heart is the most practical method of dealing with the problem of intractable angina pectoris, several other methods have been tried but have fallen into disuse because of their prohibitive mortality rate or inconstant results. These consist of:

1) Total thyroidectomy: This operation, proposed by Blumgart, Levine, and Berlin (45), is based on the reduction in work of the heart which results from lowering the basal metabolism. According to Cutler and Hoerr (46) and Blumgart (47) the operative mortality is about 9 per cent. Relief from pain is not as consistent as after interruption of the sensory nerves from the heart, as in the Peter Bent Brigham Hospital series only 8 out of 12 patients had a "sustained clinical improvement." In the single example which we have been able to observe, a patient who required further treatment after total thyroidectomy performed at another hospital, angina continued to be severe unless the basal metabolism was allowed to fall to the level of severe myxoedema. As total thyroidectomy is just as serious a procedure as sensory denervation of the heart, we no longer consider it to be a justifiable procedure in the treatment of angina pectoris.

2) Increase in collateral coronary circulation by vascular grafts: Beck's (45) method of increasing the myocardial circulation by application of vascular grafts from the intercostal muscles at first seemed to be a valuable suggestion.

* Inasmuch as Fauteux's operation requires an exposure very similar to that described by Beck (44) for his myocardial grafts, one might logically expect a very much higher mortality rate.

Continued experience, however, has shown that the mortality rate of 37.8 per cent is prohibitive (Feil (48)). Furthermore, it is highly questionable whether a muscle which no longer fulfills its normal function can retain a satisfactory circulation. On this basis O'Shaughnessy's (49) proposal of utilizing omental grafts seems distinctly more logical, and it is a pity that his studies were cut short by his untimely death at Dunkirk.

Even if the technical difficulties of attaching vascular grafts to the myocardium could be successfully solved, it remains a question if the collateral circulation could be increased rapidly enough to save many of the patients with severe angina pectoris. In the majority of these most critical cases, as we have shown above, it appears that a fairly adequate collateral circulation can develop spontaneously if the patient is relieved even temporarily from the fear and exhaustion connected with recurrent agonizing attacks of pain.

VI. SUMMARY AND CONCLUSIONS

1. All afferent painful impulses from the heart are carried to the paravertebral sympathetic chains over either the middle and inferior cardiac nerves, or over accessory cardiac rami which run to the upper three thoracic ganglia. As there are no direct connections between the cervical ganglia and the spinal cord in the neck, these pathways are all concentrated in the upper thorax, where they reach the highest three or four thoracic spinal nerves over the sympathetic rami communicantes. They then enter the spinal cord over the posterior roots. These upper thoracic ganglia, their connecting white rami communicantes, and the posterior spinal roots are the logical points for attack by the surgeon in the intractable forms of angina pectoris.

2. The pathways of cardiac pain may be interrupted effectively by chemical destruction of the sympathetic ganglia and their rami with alcohol, by sympathetic ganglionectomy, or by posterior rhizotomy. After these operations there is occasional persistence of mild pain referred to the jaw, the mechanism of which is still unknown.

3. Clinical statistics are presented which indicate that all suitable cases of angina pectoris can be relieved of their crushing precordial and arm pain by properly executed neurosurgical procedures. These patients do not lose the danger signal which warns against over-exertion, but it is no longer agonizing pain. In skilled hands surgical intervention is no more dangerous than any of the other common major operations in this age group. Once relieved of their pain, these patients are able to live happier and more active lives, free from fear of ever-recurring attacks and less conscious of impending death. They are no longer threatened with addiction to morphine, lack of sleep, and worry, which so often are factors accelerating termination of life by coronary occlusion. If fatal coronary infarction takes place, it is usually painless.

4. The relative effectiveness and risk of these procedures are evaluated by statistics drawn from 83 cases. The open surgical approach gives nearly perfect relief of pain, but cannot be used safely in patients with the most advanced forms of coronary disease. Paravertebral block with alcohol, on the other hand,

is the safest method, but fails to bring about effective denervation in a small proportion of cases (8 per cent). It is also often followed by intercostal neuralgia, which is a cause of major complaint in some 10 per cent of cases. This method must therefore be reserved for patients who are unfavourable surgical risks, but it has given striking benefit in many of this group.

5. It is recommended that surgical intervention be considered more often when adequate medical measures fail to control severe and protracted attacks of angina pectoris. With relief of pain the associated nervous tension is reduced. More complete relaxation and sleep are ensured, and the patient is thereby given his best chance of surviving a period of critical coronary insufficiency until there is spontaneous improvement in myocardial circulation by the slow development of competent collateral vessels. Such a sequence appears to have occurred in a number of our recorded cases.

Acknowledgment

We wish to express our gratitude to Dr. P. D. White, who first suggested this investigation and has constantly encouraged it; also to Dr. W. J. Mixter, who performed some of the earlier injections and operations and has given much helpful advice.

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CINCHOPHEN

(Atophan)

A CRITICAL REVIEW

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A. HISTORICAL ASPECTS

phen (2-phenyl quinoline-4-carboxylic acid) was synthesized by Giesecke (84) in 1887 who obtained this chemical as a condensation product by boiling for 3 hours molecular amounts of anilin, pyruvic acid and sodium acetate in absolute alcohol on a water bath. Numerous patents were issued on variations of this basic procedure by subsequent investigators. Development of different methods of production, such as that of Pfizinger who condensed atin and acetophane or isatinic acid and acetophenon, was greatly stimulated by the commercial importance which this chemical obtained at the turn of the century. Following Nicolaier and Dohrn's (254) discovery in 1908 that cinchophen considerably increases the excretion of uric acid in the urine, Weintraud (382) introduced it in 1911 as a drug into medicine for the treatment of gout. Because of this therapeutic use, cinchophen was known under the name of "atophan", by which it is still known in Europe. In the New World it has been replaced by the chemical term of "cinchophen". Some of the "atophan" or parts of it have been incorporated into the proprietary names of derivatives of cinchophen or of its mixtures with other drugs (novatophan, isatophan, atochinol, lytophan, hexophan, quinophan, irriatophan, etc.).

The therapeutic indication for cinchophen was subsequently extended out to a large number of other and unrelated disease conditions, such as acute polyarthritides, infectious diseases of various kinds, neuritis, headache, scurvy, and eczema, numerous derivatives of cinchophen were prepared in an attempt to improve or modify its medicinal properties and its toxic qualities. Cinchophen, or one of its derivatives, became an ingredient of many patent medicines recommended for the treatment of colds, neuralgias, rheumatism, and headache (164). These products were offered to the public under trade names and with highly exaggerated claims as to the therapeutic efficacy. This indiscriminate promotion of the sale of cinchophen was in part essentially the result of the rather enthusiastic reception which this drug received by the medical profession, particularly in Germany. This development, however, was aided by the fact that during the first twenty years of its medicinal use cinchophen apparently did not give rise to any serious toxic complications. From some occasional, minor and transitory skin reactions and gastrointestinal disturbances, the drug appeared to be practically harmless when given in therapeutic doses. The annual production of this drug by pharmaceutical companies in America and Europe reached high levels during this period (46).

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When the therapeutic indication for cinchophen was subsequently extended from gout to a large number of other and unrelated disease conditions, such as rheumatic polyarthritis, infectious diseases of various kinds, neuritis, headache, colds, jaundice, and eczema, numerous derivatives of cinchophen were prepared in an attempt to improve or modify its medicinal properties and its toxic qualities. Cinchophen, or one of its derivatives, became an ingredient of many patent medicines recommended for the treatment of colds, neuralgias, rheumatism, grippe, and headache (164). These products were offered to the public under phantastic names and with highly exaggerated claims as to the therapeutic efficacy (164). This indiscriminate promotion of the sale of cinchophen was in part doubtlessly the result of the rather enthusiastic reception which this drug received by the medical profession, particularly in Germany. This development, moreover, was aided by the fact that during the first twenty years of its medicinal use cinchophen apparently did not give rise to any serious toxic complications. Apart from some occasional, minor and transitory skin reactions and gastrointestinal disturbances, the drug appeared to be practically harmless when given in therapeutic doses. The annual production of this drug by pharmaceutical houses in America and Europe reached high levels during this period (46).

The first report on the occurrence of a serious complication in the form of an acute yellow atrophy of the liver following the administration of cinchophen did not attract much attention at the time of its publication in 1923. Subsequent communications dealing with similar observations made during this decade remained rare events and originated in the majority of cases from Anglo-American countries. Doubts as to the completely innocuous nature of cinchophen were in general during this period very mild or nonexistent.

These isolated reports, however, aroused the medical profession to assume a more critical attitude toward the drug. The appearance of jaundice and the development of acute degenerative hepatitis after the administration of cinchophen or one of its derivatives formed the subject of an increasing number of publications during the latter part of the second decade and the early part of the third decade of this century. As a significant portion of these untoward reactions attributed to cinchophen had a fatal outcome, doubts were voiced as to whether the continued use of this drug was still justified. Some investigators have gone so far as to brand cinchophen as a dangerous drug, the employment of which in medical practice should be abandoned. Others advocated a strictly controlled dispensation of cinchophen restricted to the treatment of gout when all other therapeutic measures have failed. There exists finally a group of competent American and European physicians possessing extensive experience with this drug who have not hesitated to challenge the validity of the various allegations and condemnations advanced, since they are not supported by the observations which these investigators have made with the drug over a period of many years and on a large number of patients. As the result of the present controversial status of cinchophen as a medicinal agent, the production and the therapeutic use of this chemical have considerably decreased in recent years.

Observations and studies of recent years have appreciably extended our knowledge on toxic reactions from chemotherapeutic agents and on the etiology of acute degenerative hepatitis due to chemical or infectious causes. It is the purpose of this review to present and analyze the available and pertinent data on the chemical, pharmacologic, therapeutic and toxic properties of cinchophen and its derivatives as they appear from experimental and clinical observations, and to integrate them with the existing information on drug reactions and degenerative liver disease. It is believed that through this procedure a fair and rational appraisal of the position which cinchophen and its derivatives may occupy as medicinal agents can be made.

B. PHYSICAL AND CHEMICAL PROPERTIES

Cinchophen (α -phenyl cinchoninic acid— $C_6H_5C_9H_5N \cdot COOH$) crystallizes in small colorless needles which melt at 208° – $209^\circ C$ (254) or at 212° – $213^\circ C$ (375) and which have a bitter taste. It also forms a white, odorless powder which, during the first decade after the introduction of cinchophen into medicine, often had a yellowish tint due to the presence of impurities. Cinchophen is insoluble in cold water, almost insoluble in hot water, soluble in alkalis after heating thereby forming soluble salts (sodium bicarbonate, hexamethylene tetramine),

soluble in boiling alcohol (20 parts), in boiling acetone (25 parts), in boiling benzene (250 parts), in boiling glacial acetic acid, and not readily soluble in ether. When added to a 15 per cent sodium hydroxide solution, cinchophen is temporarily dissolved, but subsequently solidifies into a thick paste through the precipitation of very fine needles of its sodium salt. When heated well above the melting point cinchophen is decomposed.

Neocinchophen (novatophan, tolysin— $\text{CH}_3\text{C}_6\text{H}_4\text{C}_6\text{H}_4\text{COOC}_2\text{H}_5$) is the ethyl ester of para-methylphenyl cinchoninic acid and is medicinally the most important of the many derivatives prepared from cinchophen. It is a yellowish white, odorless, practically tasteless, crystalline powder which is nearly insoluble in water and dilute alkalis, but is readily soluble in hot alcohol, strong acids, ether, chloroform and other lipid solvents.

A test for the qualitative identification of cinchophen and neocinchophen was described by Ekkert (96). When to 0.01 to 0.02 gm. of cinchophen or neocinchophen a few drops of concentrated hydrochloric acid and equal amounts of either alpha or beta naphthol are added, a blood red liquid is formed. Following the alkalization of this material by the addition of sodium hydroxide the cinchophen solution becomes clear and colorless, while the neocinchophen solution becomes first milky and then clear. Tests for the quantitative determination of cinchophen were developed by Rabak (279) and Palkin (258), and were reported in New and Nonofficial Remedies (253). Castiglioni (52) developed a method for the quantitative demonstration of cinchophen in tissues and Berman, Snapp, Atkinson and Ivy (28) described a method for the quantitative determination of cinchophen in bile. There does not seem to exist a quantitative test for determining the cinchophen content of the blood.

When it was shown that cinchophen possesses several pharmacologically important properties (uricosuric, analgesic, antipyretic, antiphlogistic, choleric), attempts were made to prepare derivatives having these qualities to an even higher degree than cinchophen and being, at the same time, less toxic. Efforts in these directions were made on an extensive scale. Waser (379) stated that more than 50 German patents were issued covering products which allegedly represented "improved" cinchophens. A research report of the Schering-Kahlbaum A. G. (303) mentioned that more than 200 derivatives of cinchophen were prepared and many of them pharmacologically tested by this organization alone. However, it is apparent that the therapeutic results obtained with these derivatives have not paid for the efforts made, as cinchophen and neocinchophen remained the medicinally best agents (118).

The research report of the Schering-Kahlbaum A. G. (303) listed the following derivatives: quinoline-4-carboxylic acid; 4-methylquinoline-4-carboxylic acid; 2-methylquinoline-3-carboxylic acid; 2-isopropylquinoline-4-carboxylic acid; 2-isobutylquinoline-4-carboxylic acid; 2-n-hexylquinoline-4-carboxylic acid; quinoline-2,4-dicarboxylic acid; 2,3 dimethylquinoline-4-carboxylic acid; 2,4-dimethylquinoline-4-carboxylic acid; 2-methylquinoline-3,4-dicarboxylic acid; 2,6-dimethylquinoline-3,4-dicarboxylic acid; 2-furylquinoline-4-carboxylic acid; 2-styryl-quinoline-4-carboxylic acid;

2-cyclohexylquinoline-4-carboxylic acid; 2-methyl-pyridylquinoline-4-carboxylic acid; 2-a-naphthylquinoline-4-carboxylic acid; 2-4-naphthylquinoline-4-carboxylic acid; 6-oxy-cinchophen; 8-oxy-cinchophen and its methyl and acyl derivatives (the methyl derivative known as isatophan); 2-m-p-dimethoxyphenylquinoline-3-carboxylic acid; 2-piperonylquinoline-4-carboxylic acid; 2-oxy-3-phenylquinoline-4-carboxylic acid; sulfophenyl-quinoline-4-carboxylic acid; 2-(p-oxyphenyl)quinoline-3,4-dicarboxylic acid; 2-phenyl-quinoline-3,4-dicarboxylic acid; 2-phenylquinoline-2°,4-dicarboxylic acid (Lytophan); chlorinated derivatives of cinchophen; p-iodophenylquinoline-4-carboxylic acid; 6-methylcinchophen; 8-methylcinchophen; 7-methylcinchophen; 2-benzyl-3-phenylquinoline-4-carboxylic acid; 2,3-diphenylquinoline-4-carboxylic acid; aminocinchophens with the amino group either in the quinoline nucleus or in the phenyl side group; acylated aminocinchophens.

The methyl-, ethyl-, glycollic acid ethyl-esters, amines, urea- and carbamic acid esters of cinchophen were found to be little soluble in water.

Von Oettingen (375) published the following list of the best known derivatives with their proprietary names (Table 1). In a second compilation (Table 2) this investigator presented a large number of cinchophen derivatives arranged according to their relative uricosuric properties.

Skita (329) prepared tetrahydroatophan and decahydroatophan. The tetrahydroatophan, which is produced by the reduction of cinchophen, forms yellowish white crystals which are insoluble in water and which melt at 165°-167°C. Hartmann and Wybert (158) introduced a thiophen group in place of the phenyl group and thereby obtained thienylquinoline-4-carboxylic acid, of which they also prepared the acetic acid ester. Braun and Stuckenschmidt (42) developed tetrophan (1,2-dihydro-3,4-benzacridine-9-carboxylic acid). Lublin (220) reported the production of triatophan ($C_6H_5(C_9H_5NCOOH_3)$). Impens (185) studied 40 cinchophen derivatives.

The pharmacological testing of the various derivatives showed them to differ greatly in their biological properties depending upon their chemical structure and composition. (See Table 2) (375, 300). It was shown that the introduction of a phenyl rest into the 2-position of the quinoline carboxylic acid molecule is essential for an uricosuric effect (85). In the absence of this phenyl group or in the presence of an alkyl group in its place, there is no uricosuric, antipyretic and antiphlogistic effect. With the introduction of the first ring system (furyl, styril, cyclohexyl, methylpyridin, naphthyl) in the 2-position of the quinoline-4-carboxylic acid the compounds start to show uricosuric properties (303). In addition to the phenyl group a second substitution of the quinoline nucleus must be present, which is most effective when in the 4-position as a carboxylic group. Duplication of the phenyl rest and carboxylic group does not abolish the effectiveness of the compound as an uricosuric agent. Other factors have corresponding effects (hydroxyl-, methyl-, ortho-position of phenyl-, methoxy-group in para position, amino-group, benzoyl-amino-group, esterification with alcohol, amide formation of carboxyl group). Rotter (300) noted that the substitution of a second phenyl, ethyl, amino or hydroxyl group (but not methyl) in the quinoline

nucleus considerably lowers the uricosuric effect of the resulting compound. Triatophan was found by Lublin (220) to be ineffective as an uricosuric agent, while the ethyl ester of cinchophen (acitrin) retains this property, but does not possess antiphlogistic qualities (85).

Braun (41) and Wolff (397) maintained that the action of cinchophen is conditioned by the gamma pyridin carboxylic acid located between a benzol nucleus

TABLE 1 (375)

PROPRIETARY NAME	CHEMICAL NAME
Cinchophen	2-Phenylquinoline-4-carboxylic acid
Atophan K	2-Phenylquinoline-4-carboxyl-methylester
Acetrine (Novatophan Improved)	2-Phenylquinoline-4-carboxyl-ethylester
Atoehinol	2-Phenylquinoline-4-carboxyl-allylester
Paratophan	2-Phenyl-6-methylquinoline-4-carboxylic acid
Tolysin	
Novatophan	2-Phenyl-6-methylquinoline-4-carboxyl-ethylester
Neocinchophen	
Synthaline	2-Piperonylquinoline-4-carboxyl-methylester
Lytophan	2-(o-Carboxyphenyl)-4-quinoline-carboxylic acid
Hexophan	2-(p-Hydroxy-m-carboxyphenyl)-quinoline-4-carboxylic acid
Guphen	2-Phenylquinoline-4-carboxyl-guaiacolester
Cinchosal	Ethyl-2-phenyleinchoninyl-salicylate
Diapurine	2-Phenyl-beta-naphthol-quinoline-4-carboxylic acid
Farastan	N-Iodo-2-phenylquinoline-4-carboxylic acid
Biloptin	2-(p-Iodophenyl)-6-iodoquinoline-4-carboxylic acid
Oxylodide	Hydroiodide of cinchophen
Quinophan	Brand of cinchophen
Agotan	Brand of cinchophen
Phenoquine	Brand of cinchophen
Leucotrophine	2-Phenylquinoline-4-carboxylate of methenamine
Irriphan	Strontium salt of cinchophen
Atophanyl	Solution of cinchophensodium and sodium salicylate (10% with addition of 0.16% of p-aminoethanol hydrochloride)
Eryeon	2-(p-Methoxyphenyl)-4-quinoline-carboxylic acid
Fantan	2-Phenyl-einchopinylurethane
Esophan	2-Phenyl-6-hydroxy-3,4-quinoline-dicarboxylic acid
Isatophan	2-Phenyl-8-methoxy-quinoline-4-carboxylic acid

and a hydrated or nonhydrated naphthalene nucleus. In the report of Schering-Kahlbaum (303) it is noted that oxy-groups in the phenyl radical destroy in general the uric acid eliminating properties. Only when the oxy-group is in 2-position is this quality retained. Antipyretic properties are weak and antiphlogistic properties are absent in these compounds. Oxy-cinchophens are moderately to strongly uricosuric and moderately antiphlogistic. 2-piperonyl

quinoline-4-carboxylic acid is not uricosuric, is weakly antiphlogistic and is as toxic as cinchophen. The introduction of a second carboxyl group considerably reduces the toxicity as well as the therapeutic efficacy of such chemicals. Chlorinated cinchophens with the Cl in the phenyl group are as toxic as cinchophen, less antiphlogistic, but possibly more strongly uricosuric than cinchophen.

TABLE 2 (375)

EFFECTIVE	INEFFECTIVE
2-Phenylquinoline-4-carboxylic acid	2-Phenylquinoline
2-Phenylquinoline-4-carboxylic acid ethyl	Di-hydroxyquinoline
2-Phenylquinoline-4-carboxyl-amide	Quinoline-4-carboxylic acid
2,3-Diphenylquinoline-4-carboxylic acid	Quinoline-2,4-dicarboxylic acid
2-Phenyl-6-methylquinoline-4-carboxylic acid	2-Methylquinoline-4-carboxylic acid
2-Hydroxyphenylquinoline-4-carboxylic acid	2-Methylquinoline-3-carboxylic acid
2-Phenyl-3-hydroxyquinoline-4-carboxylic acid	2-Methylquinoline-3,4-dicarboxylic acid
2-Phenylquinoline-4-carboxylic acid acetol ester	2,3-Dimethylquinoline-4-carboxylic acid
	2,3-Dimethylquinoline-3,4-dicarboxylic acid
	2-Phenyl-6-hydroxyquinoline-4,8-dicarboxylic acid
	2-Phenyl-6-methoxyquinoline-4-carboxylic acid
	2-(Dihydroxyphenyl)-quinoline-4-carboxylic acid
	2-Phenyl-6-benzoylaminoquinoline-4-carboxylic acid
	2-Phenyl-7-methylquinoline-4-carboxylic acid
	2-Phenyl-8-carboxylic acid ethylesterquinoline-4-carboxylic acid
	2-(P-methoxyphenyl)-3-phenylquinoline-4-carboxylic acid
	2-(P-tolyl)-quinoline-4-carboxylic acid
	2-(P-methoxyphenyl)-quinoline-4-carboxylic acid
	2,3-Diphenylquinoline-4-carboxylamide
	2-Phenylquinoline-4-carboxylic acid urea
	2-Phenylquinoline-4-carboxylic acid-salicylic acid ester
	2-Phenylquinoline-4-carboxylic acid ester of ethyleneglycol mono salicylate
	2-Phenylquinoline-4-carboxylic acid ethanol amide
LESS EFFECTIVE	
2-Phenyl-6-aminoquinoline-4-carboxylic acid	
2-Phenylquinoline-4,8-dicarboxylic acid	
2-Phenylquinoline-3,4-dicarboxylic acid	
2-(0-hydroxyphenyl)-7-methylquinoline-4-carboxylic acid	
2-Phenyl-8-methoxyquinoline-4-carboxylic acid	
Anhydride of 2-phenylquinoline-4-carboxylic acid	
2-Phenyl-3-ethylquinoline-4-carboxylic acid	
2-Phenylquinoline-4-carboxylic acid phenyl ester	
2-Phenylquinoline-4-carboxyl-cyclohexanol ester	

Halogens in the quinoline ring destroy the uricosuric effect. Alkylated cinchophens have good antiphlogistic properties, but usually low uricosuric ones. Benzyl cinchophens are good uricosuric agents, but weak antiphlogistic ones. Poorly soluble cinchophens have in general weak antiphlogistic and antipyretic properties or none at all.

Thienylquinoline carbonic acid, which was prepared to accentuate the anti-phlogistic and analgesic properties of cinchophen, displays marked coloring properties when injected into or fed to animals which assume a rather persistent violet red color of the skin, mucous membranes, cartilage and internal organs through the formation of a metabolite of unknown chemical composition.

Meidner (232) remarked that isatophan (2-phenyl-9-methoxy-quinoline-4-carboxylic acid), like neocinchophen, lacks the bad taste of cinchophen.

It may be mentioned that atophanyl is not a derivative of cinchophen, but a solution of sodium cinchophen, sodium salicylate and novocain (354), intended for intramuscular and intravenous injection.

C. PHARMACOLOGIC PROPERTIES

1. URIC ACID METABOLISM

I. Urine

The definite increase in the urinary excretion of uric acid following the administration of cinchophen was the reason that this chemical was introduced into medicine (254, 85, 382). Weintraud (382) noted that after the ingestion of 0.25 to 0.5 gm. of cinchophen by normal individuals there developed within one hour a definite uricosuria which regressed to its original level within 8 hours, and which was followed by a reduced excretion of uric acid during the subsequent hours. When up to 5 gms. of the drug was given in divided doses, within 24 hours the uric acid excretion was doubled or tripled. This increase lasted for 24 hours and again was followed by a phase of decreased uric acid elimination (220). These observations were confirmed by many investigators and were found to apply to gouty persons who have usually an elevated uric acid level of the blood to an even more marked degree (191, 342, and 367), as the uricosuric action in gouty patients was more pronounced and more prolonged than in normal ones (290, 191).

When cinchophen medication is kept up for several days the uricosuric effect gradually declines after having reached its peak on the second day and returns after the third or fourth day of medication to its original level. After the arrest of the administration of cinchophen the uric acid excretion drops rapidly below this level and returns several days later to normal values (342). These fluctuations are observed in man receiving a purin free diet and represent the effects of cinchophen upon the endogenous uric acid metabolism. The uricosuric action of cinchophen is not the result of nor is it associated with a diuretic effect of the drug (342). The uricosuric effect of cinchophen upon normal and gouty individuals kept on a purin free diet does not increase parallel with the dose of the drug given (290).

It is noteworthy, however, that this specific action of cinchophen upon the urinary uric acid elimination is not a consistent phenomenon. After administering 3 gms. of cinchophen to 16 middle-aged adults with normal renal function who had been given a purin free diet, Grabfield and Pratt (133) found that an elevated elimination of uric acid occurred in only 13, while in 3 there were no

changes in uric acid excretion after having been on cinchophen medication for 2 to 6 days. This observation is of clinical importance as Klemperer (196) has maintained that the beneficial effect of cinchophen in gout occurs even in the absence of an increased uricolysis, as non-uricolytic derivatives of cinchophen (7-methyl-ethyl ester of cinchophen, 6-amino cinchophen, cinchophen sulfonic acid, ethyl ester of piperonyl cinchophen) are effective in ameliorating the gouty manifestations.

The uricosuric action of cinchophen extends to the exogenous uric acid metabolism as well, since uric acid introduced into the body either by the oral or venous route or incorporated in the food in the form of its metabolic precursor, nucleic acid, is excreted more rapidly under the influence of cinchophen than when this drug is not given (367, 112, 19, 342, 311, 369). Uric acid injected intravenously is as a rule completely excreted within 2-4 days (298). Weintraud (382) as well as Bauch (19) showed that this reaction is completed within 24 hours, when cinchophen is given. Griesbach (143) noted that sometimes even more uric acid is excreted during this period than the amount administered. Schittenhelm and Ullmann (308) reported that the administration of alpha-thymonucleinate and cinchophen resulted in man in only a minor increase in uric acid excretion, suggesting that the total nucleic acid is retained in the body. In subsequent experiments on patients with rheumatic polyarthritis, arthritis deformans, and neurasthenia, these investigators observed that the simultaneous introduction of nucleic acid and cinchophen caused a marked increase in the uric acid excretion.

Frank and Przedborski (114), on the other hand, noted that the feeding of nucleic acid or hypoxanthin (0.75 gm.) and cinchophen (3 gm.) to man resulted, in only 50 per cent of the tested individuals, in an increased excretion (50 to 300 per cent) of uric acid. These observations contrast with those made by Bauch (19) in gouty persons and by Deutsch (81) in normal ones subjected to similar experimental conditions. In these individuals they found a consistent increase in uric acid elimination. Griesbach and Sampson (145) pointed out that persons receiving little or no meat over long periods of time do not react with an uricosuria after cinchophen medication, as their endogenous uric acid excretion represents the maximum values possible. Man kept on a purin free diet does not excrete any allantoin when given cinchophen (144). It is, therefore, unlikely that cinchophen has any effect upon uric acid oxidation in man.

While a great deal of work has been done on experimental animals in studying the uricosuric effect of cinchophen and its underlying mechanism, there exist some fundamental differences in uric acid metabolism between animals and man which interfere with the direct application of the findings made on experimental animals to the reactions observed in man. In dogs the final metabolite of purin is allantoin, an oxidation product of uric acid. Inasmuch as uric acid excreted by dogs may be entirely of exogenous origin, these animals are not well suited for studying the uricosuric effect of cinchophen (131). Starkenstein (342) noted in dogs on a purin free diet that after cinchophen medication there was a decrease in the allantoin excretion and a simultaneous minor increase of uric acid elimination. He concluded from this observation that in dogs cinchophen inhibits uric acid

oxidation. Frommherz (119) found in two dogs after the administration of cinchophen an increase of allantoin excretion, while in a third dog there was a marked reduction in the elimination of this substance.

Schüttenhelm and Ullmann (308), on the other hand, did not notice any effect upon the allantoin content of the urine after the administration of cinchophen to dogs. Boenheim (31), contending that an uricosuric effect of cinchophen in dogs should be manifested by an increase in allantoin excretion and a decrease of uric acid elimination, found that cinchosal (phenyleinchonin salicylic ethyl ester) reduced the excretion of uric acid and increased that of allantoin. Acetyl salicylic acid-8-oxyquinoline and oxyquinoline hydrochloride exerted a similar effect, but the last-mentioned chemical proved to be highly toxic. Bass (17) mentioned that a typical cinchophen effect was not demonstrable in dogs. Grabfield and Swanson (135), however, stated that dogs receiving 300 mg. of cinchophen showed an increased excretion of uric acid, like man. Starkenstein (342) as well as Frommherz (119) noted in purin free maintained dogs a decrease in the urinary purin content after the administration of cinchophen, which was associated with a reduced excretion of allantoin and a throttling of the entire purin metabolism.

Nagashima (248) concluded that cinchophen decreases the excretion of allantoin. Grabfield (131) observed in Dalmatian dogs, which have a metabolic anomaly in the form of a deficiency of the normal oxidation of uric acid and which, therefore, stand metabolically in this respect between man and normal dogs, an increased excretion of uric acid and allantoin following the administration of cinchophen. Grabfield (131) took this finding as additional evidence that cinchophen acts in the same way upon both the endogenous and exogenous uric acid metabolism in dogs. Frommherz (119) observed that dogs fed horse meat showed a doubling of allantoin excretion after oral administration of cinchophen. A similar effect with regard to allantoin excretion was elicited in dogs kept on a purin free diet and given cinchophen, while there was no change in the uric acid elimination. In subsequent experiments Frommherz (119) found that cinchophen decreased the allantoin excretion, thereby confirming Starkenstein (342). Frommherz (199) concluded from this evidence that cinchophen does not inhibit uric acid oxidation.

There is no evidence in the dog that nucleic acid degradation is enhanced by cinchophen. Dogs and rabbits fed sodium nucleinate do not excrete more purin metabolites when given cinchophen, according to Starkenstein (342). Schroeder (311) reported that dogs given 2 gms. of cinchophen by mouth and injected some time later with uric acid by vein revealed a 100 per cent elevation in the excretion of uric acid. Chickens, which, like man, excrete uric acid as the metabolic end-product of purin decomposition, exhibit after cinchophen administration a decrease of uric acid excretion and an increase of urea elimination, indicating that a disturbance in uric acid synthesis is produced (342). Rabbits show after cinchophen medication an increase in the excretion of allantoin and uric acid (342, 311). Dohrn (85) found the excretion of allantoin in rabbits reduced after cinchophen medication.

It is obvious that no definite conclusions can be drawn from these partly

contradictory observations made on experimental animals as to the uricosuric mechanism elicited in man by cinchophen.

In addition to the changes in the uric acid content of the urine, the following effects of cinchophen upon the composition of this excretory product were observed. Kehrner (191) maintained on the basis of insufficient evidence that the uricosuria after cinchophen medication is associated with a 10 per cent decrease in the purin bases excreted. Denis (76) noted that cinchophen has no effect upon creatin output. There are no changes in the phosphoric acid content of the urine (85, 382, 296), but the neutral sulfur fraction is increased (331), pointing to a reduced oxidative metabolism (342). However, Weintraud did not find any changes in the total sulfur excretion.

Skorczewski (330) and Skorczewski and Sohn (331) described several color tests for the demonstration of one of the urinary metabolites of cinchophen, oxycinchophen. This has an OH group in place of an H group in the benzol ring. When cinchophen urine is treated with ammonium sulfate and ammonia is then slowly added a green color develops. Greinert (142) observed that the dark green color reaction elicited in the urine by the addition of ammonium sulfate and ammonia appears first 2 hours after the administration of cinchophen. The addition of phosphotungstic acid to cinchophen urine elicits the development of a yellow precipitate, which is soluble in alkalis. The filtrate of such urine gives a typical diazo reaction. This reaction decreases in intensity with increasing length of cinchophen medication until it finally becomes negative. Lichtman (214) elaborated on these observations and developed a sensitive quantitative colorimetric method for the estimation of 2-(ortho-hydroxy-phenyl)-quinoline-4-carboxylic acid (oxy-cinchophen). The test is so sensitive that it demonstrates the presence of this chemical in a concentration of 0.0002 per cent in the urine. The test is intended as a part of a liver function test in which the metabolization of cinchophen by the liver is measured. Greinert (142) attributed the positive diazo reaction in cinchophen urine to the presence of a metabolite of this compound. Since the diazo reaction is generally caused by the presence of urochromogen beta, the diazo reaction of cinchophen urine is not a typical one. The ether extract of cinchophen urine gives a strong diazo reaction. The reaction is not interfered with by salicylates, neo-cinchophen and related compounds. Some cinchophen urines give a positive diazo reaction only after incubation for a period of 24 hours. The gradual disappearance of the diazo reaction following prolonged medication with cinchophen indicates that the metabolization of this chemical in the body undergoes some radical changes (330).

Cinchophen urine freshly voided has a citrus yellow color which turns brown on standing. The studies of Scheunemann (304) on the behaviour of quinolines in the animal body make it probable that there occur other urinary metabolites beside 8-oxy-2-phenylquinoline-4-carboxylic acid (85). This investigator suggested that one of these compounds is a hydroxylated substance (oxypyridinuric acid), while a third one is of unknown composition and is scarcely soluble in glacial acetic acid.

Rotter (300) extracted from human cinchophen urine a metabolite of this chemical, which was obtained as longitudinal, small, greenish-yellow crystals in brush-like arrangement, having a melting point of 232°C . It is readily soluble in ethyl alcohol, not in petroleum ether, slightly in benzol or water, moderately in ether and methyl alcohol. It is probably 2-o-oxyphenyl quinoline-4-carboxylic acid and is toxic to frogs. A second metabolite extracted with ether from human cinchophen urine which does not give a positive diazo reaction is non-toxic to frogs. If the urine of dogs was extracted after the administration of cinchophen, a brown red substance was isolated which was non-toxic to frogs, and which did not give a positive diazo reaction. Thus the urinary metabolites of dogs differ from those of man.

There are no changes in the pH of the urine (223). The reduction test is rarely positive. The titrable reducing power of the urine is appreciably increased. The chemical nature of this reducing substance is unknown. Its reducing properties are lowered on exposure to air in the presence of a catalytic agent such as copper (223).

II. Blood

The fluctuations of the urinary content of uric acid have been correlated with the changes in the blood uric acid level observed after the administration of cinchophen. However, the observations made on this aspect were quite contradictory and unreliable, until Folin and Denis (109) developed in 1913 an accurate method for the determination of uric acid in the blood.

Kehrer (191) reported an increase in the uric acid content of the blood after cinchophen medication (1913). Retzlaff (290) in 1914 recorded a similar reaction when 2 gms. of cinchophen were given to normal individuals whose uric acid level of the blood allegedly increased from zero before the cinchophen medication to 1.5 to 3.8 mg. per 100 cc. after it. In gouty persons, on the other hand, he confirmed the previous findings of Zuelzer (403) who in 1911 reported a decrease of the uric acid level of the blood following the administration of cinchophen. Deutsch (81) did not see any uric acid in the blood when cinchophen was given over prolonged periods. A reduction in the uric acid content of the blood following cinchophen medication to persons suffering from gout was reported in 1913 by MacLester (227), in 1912 by Dohrn (85), and in the same year by Frank (111). Wiechowski (392) and Bass (17) in 1912, on the other hand, noted after cinchophen medication an increase in the uric acid level. The variations in these results are largely attributable to the use of unsuitable analytical methods.

Folin and Lyman (110) in 1913 found, using their own method, a decrease of the uric acid content of the blood in gouty persons after cinchophen medication. Fine and Chace (104) studying 12 normal, arthritic, or gouty persons observed after the administration of cinchophen a drop of the blood uric acid level from 2.0 to 5.7 mg. per cent to 0.7 to 2.0 mg. per cent. The daily introduction of 4 gms. of cinchophen for 45 days did not lower the blood uric acid level below 0.7 mg. per cent. Bass (17) who gave 1-3 gms. of cinchophen to normal men

and studied the blood 2-3 hours later, noted after a single dose of cinchophen in only one out of 6 persons a lowering of the blood uric acid content, while there was no change in this factor in the other 5 persons. Prolonged cinchophen medication, on the other hand, regularly reduced the uric acid level. Frank and Pietrulla (113) observed a lowering of the uric acid content of the blood in 7 normal persons, 4 with renal disease and 2 with gout. After ceasing the cinchophen medication, abnormally high uric acid values were obtained. These observations made on patients with renal diseases by Frank and Pietrulla are of interest, as Fine and Chace (104) recorded in 6 cases of nephritis treated with 2-6 gms. of cinchophen a lessened uricosuric effect of the drug, which they attributed to functional changes of the renal cells. Frank and Pietrulla reported, moreover, that there was no change in the uric acid level 30 minutes after the administration of cinchophen when an increase in the urinary excretion of uric acid was already demonstrable. The uric acid content of the blood dropped after 2 hours and remained low for 24 hours (113, 110). Such reductions in the uric acid level of the blood were observed with prolonged cinchophen medication only when a special purin free diet was given, as there was a rise in the uric acid content of the blood in persons kept on a diet containing purin (145). A reduction in the uric acid content of the blood after the administration of cinchophen and/or neocinchophen was reported also by Smith and Hawk (332), Myers, Killian and Simpson (247), Myers and Killian (246), and Denis (76).

Contrasting observations were made by Grabfield and Pratt (133), Wolff (397), and Griesbach and Samson (145). Grabfield and Pratt (133) noted that only one out of nine persons given cinchophen showed a reduction of the blood uric acid level. Three hours after the medication there was even a definite rise. Griesbach and Samson (145) recorded a similar observation, but found that the primary elevation of the uric acid values was followed by a drop below the original level. The primary reaction of uric acid increase was more prolonged in persons who were not kept on a purin free diet. Griesbach and Costopanagiotis (144) noted that the uricosuric response was preceded by an elevation of the blood uric acid values. Wolff (397) found a rise in the blood uric acid content 20 minutes after the intravenous injection of 0.5 to 2.0 cc. of a 10 per cent sodium cinchophen solution in patients receiving a purin free diet. The increase in blood uric acid and urinary uric acid after the prolonged administration of cinchophen ceased with the exhaustion of the tissue depots of uric acid mobilized under the influence of cinchophen (144). Grabfield and Pratt (133) concluded from the fact that there was not a constant drop of the blood uric acid content in the presence of a definite uricosuric effect, that cinchophen must act not only on the renal function but also on the tissues. Myers, Killian and Simpson (247) remarked that marked variations occur in different individuals in the degree of decrease of the blood uric acid content following the administration of cinchophen.

Correlating the total amount of uric acid present in the blood of an adult man with the quantity of this metabolite excreted with the urine following the administration of cinchophen, Graham (138) concluded that the blood does not

contain sufficient amounts of uric acid to account for the increased elimination of this substance with the urine. As the blood thus cannot be the source of the uric acid excreted in cinchophen uricosuria, the tissue fluids must furnish this material.

Piung-Hun-Ri (269), using rabbits intravenously injected with Erycon (2-p-methoxy-phenylquinoline-4-carboxylic acid), found that the increase in the blood uric acid content did not go parallel with the urinary excretion of this metabolite. The peak of the rise in blood uric acid was seen four hours after the administration of the cinchophen derivative. The highest amounts of uric acid were present in the blood of the hepatic veins, somewhat lower ones in the blood of the portal vein, and the lowest values were obtained in the blood of the femoral veins. The discrepancies between these values were most marked during the period when the peak values were present.

It is obvious that the great majority of observers found after the administration of cinchophen a reduction of the uric acid level in the blood of man. The opposing evidence provided by several investigators suggests that this reaction may be preceded by a brief phase during which the uric acid level of the blood is elevated.

Myers and Killian (246) found that cinchophen and neocinchophen lower also the chloride and urea content of the blood, while Folin and Lyman (110) noted that cinchophen reduces non-protein nitrogen and urea whenever these substances occur in the blood in abnormally high amounts. Deussis (80) did not observe any changes in the creatin content of the blood which would have suggested a direct effect of cinchophen on the protein metabolism. Studies of Rawls (285) in man and of Hueper (182) in rats kept on a diet low in vitamin K showed that prolonged administration of cinchophen lengthens the prothrombin time and thus exerts a similar effect as that of an excessive medication with salicylates. Johnson (187) did not notice any changes in the alkali reserve of the blood of cats given cinchophen. A lowering of the quotient of total calcium to free calcium of the serum associated with an increase of the free calcium fraction was reported by Herrmann (169).

Bass (17) asserted that cinchophen given to normal men in amounts of 1-3 gms. appears in the blood as oxycinchophen.

III. Mechanism of Uricosuria

The mechanism of action of cinchophen upon the uric acid metabolism has remained controversial and the evidence offered in support of the different conceptions is not infrequently contradictory. The following conceptions have been advanced.

a. *Effect through action upon the autonomous nervous system.* Abl (1) claimed that the uricosuric action of cinchophen is the result of a stimulation of the sympathetic nerves by direct action upon the renal cells, and not indirectly through the vasomotor nerves by changes in the vascular tonus of the renal vessels. This is opposed to the view of Myers and Killian (246) who emphasized the role which the hyperemia of the glomerular capillaries might play in the

increased excretion of uric acid. Grabfield and Gray (132) noted in dogs with denervated kidneys that the uricosuric effect was lessened. These investigators concluded from this evidence that the real effect of cinchophen may be a decreased excretion of uric acid which is secondarily overcompensated by the reaction of the sympathetic nervous system through the medium of a hormonal agent. In support of this conception Grabfield, Prescott and Swan (134) pointed out that sympathetic paresis brought about by the administration of ergotamin abolishes the uricosuric effect of cinchophen and eliminates the increase of allantoin and nitrogen excretion in dogs. They confirmed thereby Harpuder's observation (157) that sympathetic paresis caused by ergotamin reduces uric acid excretion, while sympathetic stimulation increases it, possibly by the mobilization of uric acid depots of the tissues. Piung-Hun Ri (269), on the other hand, reported that the uric acid content of the blood in the hepatic and portal veins rises after the injection of erycon in bilaterally splachniectomized rabbits for a period of 4 hours and then drops slowly. The vagus thus does not exert an inhibitory action upon the uricosuric effect of cinchophen, according to Piung-Hun Ri.

Starkenstein (342) believed that the uricosuric action of cinchophen was mainly mediated by the parasympathetic nerve because calcium salts and atropin counteracted the effect of cinchophen. However, atrophin has no effect upon the uricosuric action of cinchophen, while increasing the elimination of allantoin in dogs (134). Grabfield, Prescott and Swan (134) found that adrenergic fibers of the kidney mediate the uricosuric effect of cinchophen, whereas the action on the allantoin excretion is mediated through both adrenergic and cholinergic fibers. Eisner (95) noted that the calcium effect is dissimilar from the cinchophen especially as to the antiphlogistic action of these two agents. Griesbach and Samson (145) stated that cinchophen uricosuria is not the result of a sympathetic stimulation of the intestine permitting the uric acid to enter the blood, as Schroeder and Raginsky (312) showed that the excretion of uric acid into the intestine is not affected when cinchophen or neocinchophen is injected together with uric acid.

b. Effect through action upon enzymes. Kehrer (191) asserted that cinchophen activates the purin desamidases and xanthinoxidases and thus has a ferment specific effect favoring the oxidative transformation of purin bases into uric acid. Rosenberg (297) supported such a conception when he found that purin depots are mobilized in dog's liver perfused with cinchophen solution. Retzlaff (290) drew similar conclusions by assuming that cinchophen degrades nucleoproteins. However, Dohrn (85), Weintraud (382), and Starkenstein (342) did not find an increase in the excretion of phosphoric acid, which was postulated by Biberfeld (29), and which would be associated with a metabolic decomposition of nucleoproteins. Fasiani (102), investigating this alleged effect of cinchophen on nucleoproteins, noted that the serum obtained after cinchophen administration has no effect upon nucleic acid, that cinchophen solution added to human serum containing sodium nucleinate is ineffective in causing a destruction of this chemical, and that cinchophen does not change the nuclease activity of the serum of dogs.

Starkenstein (342), on the other hand, found that cinchophen inhibits the purin formation in liver pulp and thereby impairs uric acid formation. The activity of the uric oxidase of renal pulp, however, is not interfered with by cinchophen. These observations do not confirm the claim of Skorzewski (330) that the uricosuria is the result of a disturbance of the oxidative metabolism caused by cinchophen. Schittenhelm (307) declared this speculation without actual basis of fact and Starkenstein (342) maintained that it has been proven to be incorrect as to man and not convincingly shown to be valid for animals.

Starkenstein (342) advanced the theory that cinchophen merely hastens the decomposition of those nucleoproteins which are already predestined to this fate, and that it does not favor the general degradation of purins. Schittenhelm and Ullmann (308) expressed a similar thought when they maintained that cinchophen acts on the intermediary metabolism by mobilizing nuclein rests which are rapidly decomposed to uric acid.

Related to the fermentative theories on the uricosuric action of cinchophen is the conception of Mendel (233) who advocated that cinchophen exerts a cytotoxic effect upon leucocytes, the decomposition of which furnishes the uric acid for the uricosuria. This contention is based upon the incidental observation of Mendel (233), who found a decrease in the number of leucocytes in a leukemic patient after the administration of cinchophen, which was accompanied by a definite uricosuria. Levi (212) noted that cinchophen increased the excretion of uric acid in leukemics to a lesser degree than in gouty individuals. Joël (186) observed in leukemics only a transitory increase in uric acid elimination without any changes in the number of leucocytes after cinchophen administration. Roesler and Jarczyk (296) reported uricosuria in leukemics after medication with cinchophen without any curative result and without any effect upon the nitrogen metabolism and number of leucocytes, and proposed that the uricosuria was the result of the mobilization of uric acid deposits. Schittenhelm and Ullman (308) obtained with cinchophen a decrease of leucocytes and a uricosuria in leukemic patients. Swift, Miller and Boots (359) recorded a depressing effect by neocinchophen upon the leucocytosis of rheumatic patients. Recent investigations of Hueper (182) on dogs given toxic and subtoxic doses of cinchophen did not reveal any leukopenic reactions in these animals, even after the administration of toxic doses with this agent for a period of 24 days. Starkenstein (342) finally contended that the leukopenic response observed by Mendel (233) following the injection of leukotropin is not attributable to the cinchophen present in this material but to the methenamine. Inasmuch as there is no increase of phosphoric acid and of total sulfur in the urine (382) after the administration of cinchophen, there is no convincing evidence available that this chemical exerts any direct cytotoxic effect on leucocytes or any other cells resulting in an increased protein metabolism, causing an increased production of uric acid (111, 250, 36).

c. *Effect by mobilization of uric acid depots.* Many investigators look upon the uricosuria as the result of a mobilization of preformed uric acid depots (290, 145, 211, 382, 119, 25, 112, 17, 108, 149, 110, 160, 104, 113). This uricolytic effect, however, does not necessarily include any mobilization of the uric acid

present in gouty tophi (227). Supporting this assumption is an experimental observation of Schroeder (311) who noted that the kidneys of cats intravenously injected with atophanyl and uric acid contained about 1/7th as much uric acid as the kidneys of cats which received uric acid only. It was concluded from this evidence that cinchophen mobilizes the excessive amounts of uric acid stored in the kidney of gouty persons (108). Stern (348) proposed that cinchophen enhances the solubility of uric acid in watery mediums by forming apparently a cinchophen-uric acid complex possessing increasing hydrotropic properties. This concept deserves consideration as the changes in the uric acid content of the blood following the administration of cinchophen do not favor an excretory mechanism based upon an excess of uric acid in the blood resulting from mobilized tissue uric acid.

d. Effect by increased permeability of the kidney. A direct effect of cinchophen upon the permeability of the kidney to uric acid causing an overflow of uric acid from the blood and the tissues into the urine was proposed by Starkenstein (342) Schroeder (311) MacLester (227) Folin and Lyman (110) Fine and Chace (104) Daniels (71) Weintraud (382) Tannhauser (362) Steinitz (346) and others. There is little direct evidence supporting this view. Frank (111) and Fürth and Scholl (122) found that cinchophen hastens the diffusion of dyes into gels. Faludi (100), on the other hand, noted that cinchophen does not change the velocity of ultrafiltration of serum and plasma through membranes. Goldwasser (130) noted that cinchophen has a depressing effect upon the amount of surface active substances in the urine, because cinchophen impairs the purin metabolism of the body. Urinary surface active substances, on the other hand, are increased after the administration of purin bodies.

From the evidence presented it appears that the uricosuric action of cinchophen is a rather complex phenomenon to which several factors may contribute. It is likely that a combination of autonomic and central nervous stimuli and a direct effect upon the permeability of the kidney are involved in the production of the uricosuric action of cinchophen.

2. WATER AND SUGAR METABOLISM

Georgiewsky (125) claimed that cinchophen possesses diuretic qualities; however, Starkenstein (342) pointed out that cinchophen does not initiate diuresis, but only accentuates one induced by the administration of water or salt solution given orally or intravenously to man or rabbits, or of diuretics, such as potassium acetate and theobromine. This diuretic effect apparently is due to the removal of an inhibitory renal factor related to the sympathetic nervous system, but not to the stimulation of the secretion of water. The diuretic action of adrenalin is greatly accentuated by cinchophen, while the glycosuria caused by adrenalin is not affected by cinchophen.

Cinchophen, on the other hand, inhibits the central glycosuria resulting from piqûre or suffocation. Pancreatogenic glycosuria, on the other hand, remains unchanged by cinchophen. There were no changes in the blood sugar level of dogs which received toxic doses of cinchophen (182).

The removal of fluorescein from the blood into the tissues is speeded up by cinchophen and thus its renal excretion is delayed.

3. GASTROINTESTINAL TRACT

Quantitative investigations in the absorption of cinchophen given orally to man do not exist (154). Some information in this matter, however, is available in regard to neocinchophen. Barbour and Lozinsky (11) did not recover from the feces of dogs and one man any neocinchophen when doses of 0.3 to 0.5 gm./kg. were given, indicating that the chemical was completely absorbed. When 9 gms./kg. of neocinchophen were given, however, only 2.7 gms./kg. were absorbed; the balance was found in the feces. Hanzlik (154) concluded from this evidence that the maximum limit of absorption is identical with the maximum therapeutic dose. Fuerth and Kuh (121) pointed out that neocinchophen, because it is lipid soluble, is absorbed through the intestinal wall with the fatty parts of the food. Employing dogs, one set of which received 2.0-12.0 gms. of neocinchophen with their daily food for a period of 5 days, while the second set was given 2.5 to 7.5 gms. of cinchophen daily for 2 to 4 days, Fuerth and Kuh (121) found that the intestinal absorption of both compounds was of about the same order. Three men who ingested 20 times 0.33 gm. of neocinchophen in 3.5 days (total 6.6 gms.), 4 times 0.5 gm. to 3.0 gms. in 3 days (total 6 gms.), and 5.5 gms. in 3 days, respectively, showed practically complete intestinal absorption of the drug (98-100 per cent). Fuerth and Kuh (121) concluded from these investigations that the effective doses of cinchophen and neocinchophen follow a ratio of 1:1.4.

Stalker, Bollman and Mann (341) studied the effect of cinchophen on the gastric secretion of dogs using gastric pouches for obtaining gastric juice. When 0.25 to 2 gms. of cinchophen were given daily by mouth for 11 days there was no change in gastric acidity, but there was a prolonged secretory curve for from 30 to 60 minutes. This reaction stopped when cinchophen medication was discontinued. An increase in the volume of gastric secretion developed with continued medication, but decreased after the appearance of anatomical mucosal lesions. Thus, cinchophen, like many other toxins, first stimulates and then depresses gastric function. With small doses there is a continuous hypersecretion, while with large doses this stage is followed by a reduction in the volume of secretion.

Abl (1) tried to explain the cinchophen action by a paresis of the splanchnic nerve and a stimulation of the intestinal glands. In checking this Schroeder and Raginsky (312) injected cats intravenously with uric acid and then gave cinchophen orally so as to determine the amounts of uric acid appearing in the different parts of the gastrointestinal tract. There were traces in the stomach, definite amounts in the duodenum, jejunum and ileum, and less in the colon. As cats given uric acid showed the same secretory effects, it appeared that cinchophen had no action of the sort suggested by Abl (1).

Starkenstein (342) applied directly to the isolated intestine of rabbits and guinea pigs sodium cinchophen (0.4 cc. of a 10 per cent. sol.) and leukotropin

(0.3 cc. of a 40% mixture containing 0.5 gm. of cinchophen and 1.5 gm. of methenamine in 5 cc.) and observed an immediate, fleeting and moderate stimulation followed by depression and paralysis. He concluded from this evidence that the intestinal stimulation seen in vivo could not be due to a stimulation of the vagus nerve. Ullmann (368), who applied the same solution to guinea pigs' intestine, noted a parasympathetic stimulation (increased tonus and peristalsis, antagonism to atropin), which he interpreted as evidence of a vagus shock.

Taubmann (364) found, while studying fat digestion in dogs fed olive oil, that after the intravenous administration of cinchophen the excreted bile containing cinchophen has a reduced activating effect upon the intestinal lipase when compared with normal bile. Cinchophen bile was found, moreover, to be inferior to normal bile in its emulsifying properties. Cinchophen bile slightly inhibits diastase but does not affect trypsin activity.

4. KIDNEY

Cinchophen administered in full therapeutic dose caused a diminution of the excretion of phenolsulphonphthalein in all of 5 men tested, whereas neocinchophen exerted such an action in only 50 per cent of 9 cases, when this effect was associated with albuminuria, according to Hanzlik, Scott, Weidenthal, and Fetterman (154). Eisner (95) contended that the excretion of sodium chloride and total nitrogen was reduced in eight patients given cinchophen. The appearance of albumin, leucocytes and casts in the urine of patients given large amounts of cinchophen (4-19 gms.) and neocinchophen (5-26 gms.) was reported by Hanzlik, Scott, Weidenthal and Fetterman (155). However, this is not a consistent finding, as Barbour, Lozinsky and Clement (12) did not observe such reactions in 19 patients who received 16 gms. of neocinchophen. Whenever such reactions occur, they represent toxic effects of cinchophen upon the renal parenchyma.

5. LIVER

Studying the metabolic effect of cinchophen upon the liver Lutwak-Mann (223) subjected slices, pulp and extract of rat liver to cinchophen solution of various concentrations. He found that concentrations as low as M/50 restricted the uptake of oxygen by liver tissue. The anaerobic acid production was diminished with concentrations of M/20. Rat liver did not show any ability to form conjugates with glucuronic acid. The respiration of liver slices taken from rats which had ingested 20-40 mg./kg. of cinchophen with their daily food was not affected. There was, moreover, no change in the dismutation of hexose-diphosphate and pyruvate of the muscle tissue of these rats. But the glycogen content of the liver was remarkably lowered. Cinchophen affected also the activity of indolphenol oxidase, of certain dehydrogenases and of glucose fermentation of yeast.

Mention has already been made of the observation of Rosenberg (297), who found that cinchophen perfused through the liver of dogs mobilized purins, while Starkenstein noted that cinchophen inhibited the purin formation in liver pulp.

Two pharmacological effects of cinchophen on the liver have obtained some passing practical significance in medicine, i. e., the choleretic action and the metabolism of cinchophen in the liver.

Brugsch (45) and Horsters (179) observed that cinchophen given to man and to dogs with a bile fistula increased the secretion of bile up to 100 per cent within 24 hours. The dry residue was doubled, but the bile pigments were increased only by 35 per cent, while the alcohol precipitable and acetic acid precipitable fractions were reduced. The peak of increased bile production was observed 3 to 4 hours following the intravenous injection of sodium cinchophen. When larger amounts of cinchophen were given there resulted a considerable decrease in the total amount of bile secreted which was associated with a reduction in the dry residue, the bile pigments and the alcohol and acetic acid precipitable fractions.

While the water content of the bile at the time was increased, the amount of bile acids, its specific gravity, its viscosity and its pH were lowered. Brugsch (45) and Horsters (179) attributed the choleretic effect to a direct stimulation of the functional activity of the liver cells by cinchophen. Inasmuch as the choleretic effect was absent in dogs with severe liver damage, Brugsch (45) and Horsters (179) proposed to use this hepatic reaction of cinchophen in a liver function test, and to employ a preparation composed of cinchophen and bile acids (choleretin) for the treatment of jaundice and similar hepatic conditions in which a choleretic effect seemed to be desirable.

Grunenberg and Ullman (148) confirmed these findings in man, recovering the bile by a duodenal tube and using sodium cinchophen (ikterosan) as the choleretic agent. Henius (167) reported the expulsion of a hazelnut sized gall stone after the intravenous injection of this drug. Teschenberg and Hoffman (365) recorded that atophanyl given orally was choleretically more effective than ikterosan administered intravenously. Horsters (179) noted that there existed a parallelism between the choleretic and uricosuric action of the various cinchophen derivatives tested. Schaffler (302) reported that even small doses of intravenously administered sodium cinchophen increased the bile production 5 to 6 fold within 6 hours and that the oral medication gave less satisfactory results than the parenteral one. He observed, moreover, that in jaundiced patients given cinchophen there occurred an increase in the serum bilirubin level, which Franke (115) noted even after therapeutic doses to persons with normal livers. Kurti (205) mentioned that cinchophen elicits, in addition to the choleretic effect, an augmented elimination (30-50 per cent above normal) of uric acid and cinchophen in the bile in patients with renal diseases. Chiray et al. (60) reported that sodium cinchophen intravenously injected into normal individuals markedly stimulates the pigmentary function of the liver cells, while it lowers this hepatic activity in patients with degenerative liver diseases.

Lichtman (214) found that diseased liver cells have a lowered capacity to metabolize cinchophen beyond the stage of oxycinchophen. He used this fact in the development of his liver function test previously mentioned. Following the ingestion of a standard dose of 0.45 gm. of cinchophen the amount of oxycinchophen excreted in 24 hour urine was determined. Normal persons excrete

from 30–100 mg. of oxycinchophen or 7 to 21 per cent of the administered dose, patients with moderate to mild local liver damage (biliary obstruction, metastatic carcinoma, cirrhosis, catarrhal jaundice) excrete between 100 and 200 mg. of oxycinchophen, while patients with diffuse hepatic degenerations (acute yellow atrophy, carcinoma of the pancreas) show excretory values above 200 mg.

The choleric effect of cinchophen in man and in dogs was demonstrated also by Bradley and Ivy (38); Chabrol and Maximin (56); Annegers, Snapp, Ivy and Atkinson (5); Steinmetzer (347); Berman, Snapp, Atkinson and Ivy (28); Brugsch (45); Stransky (351); Spurling and Hartman (338); Steinberg (345); Franke (115); and Faludi (100). Several of these investigators showed that large doses or repeated small doses of cinchophen inhibit the secretion of bile in dogs, cats and rabbits (115, 100, 345). Winogradow (395) brought out the fact that this reaction resembles that seen in chloroform poisoning, which becomes apparent long before the narcotic action of chloroform. Stransky (351) pointed out that numerous, but not all, narcotic agents, including cinchophen, increase the bile flow in dogs and that all of them are injurious to the liver. The choleric effect is, in his opinion, a manifestation of liver injury or of toxic irritation to the liver cells which is followed, when excessive doses are given, by a functional paresis. While Kalk (190) considered cinchophen and its derivatives not only a choleric but also a hepatotoxin when used in diseases of the liver or the bile ducts, Franke (115) felt that such a combined action might be exerted even by therapeutic doses of cinchophen. The first sign of an impaired liver function caused by cinchophen is a reduction of the amount of bile pigments excreted, according to Franke (115). Rabbits appear to be especially sensitive in this respect, as Stransky (351); Berman and Ivy (27); Steinberg (345), and Franke (115) observed either no choleric effect of cinchophen in these animals or only a minor and fleeting one, even when small doses of the drug were given.

Ivy and his coworkers (4, 5, 6, 27, 28) have studied extensively the effect of cinchophen on bile production and other hepatic functions in dogs. Using anesthetized dogs and dogs with chronic bile fistulas, which received by vein 50 mg./kg. of sodium cinchophen, Bradley and Ivy (38) determined the amounts of cinchophen, bile pigments, cholic acid and cholesterol excreted with the bile. The anesthetized dogs showed during the first hour after the cinchophen administration a choleresis associated with an increase of bile pigments, cholic acid and cholesterol. With continued choleresis a depression of the cholic acid output occurred. The peak of choleresis was during the second hour. The bile collected during 5 hours contained 97.8 mg. of cinchophen or 20 per cent of the intravenously injected 505 mg.

Biliary fistula dogs receiving a daily toxic dose of 100 mg./kg. of cinchophen with their meals showed a daily recovery of cinchophen in the bile ranging from 28 per cent to 78 per cent (average 55 per cent). The cholic acid synthesis was finally depressed, but this effect might have been due to the anorexia and vomiting existing at that phase of the experiment.

Berman, Snapp, Atkinson and Ivy (28) fed several chronic biliary fistula dogs a daily dose of 0.5 gm. of cinchophen for 3, 14 and 33 days, respectively.

There developed a marked hydrocholerisis, with no changes in the cholic acid and total bile pigment output, and an increased cholesterol excretion. Sixty to seventy-five per cent of the ingested cinchophen was recovered from the bile. In subsequent studies Annegers, Snapp, Ivy and Atkinson (5) gave to dogs 1 and 4 times the dose of cinchophen recommended for the treatment of gout and failed to cause any disturbances in the liver function demonstrable with ordinary methods. There was a marked temporary depression of synthesis of cholic acid, a reaction which, however, was completely suppressed when anorexia and other objective gastrointestinal symptoms were absent. Most of the dogs recovered from this depression with continued medication of cinchophen.

On a gram-weight basis cinchophen increased bile flow three times as effectively as dehydrocholic acid. Cinchophen did not, however, improve or aggravate the hepatitis present in one of the dogs. The liver of the dog, but not that of the rabbit, appears to be especially concerned with the excretion of cinchophen in the bile, in the opinion of Ivy and his coworkers (4, 5, 6, 27, 28, 38).

Berman, Snapp, Atkinson and Ivy (28) suggested that the ease of solubility of cinchophen in bile and its osmotic activity therein are important factors contributing to the hydrocholerisis produced in the dog. Faludi (100) stated that cinchophen must act directly on the liver cells as the ultrafiltration speed of plasma and serum are not influenced by cinchophen and bile acids. Faludi (100) noted that the cholcretic effect of cinchophen is accompanied by a decrease in the refractive value of the blood indicating a hydremia. As cinchophen and/or neocinchophen hastens the excretion of eosin and sodium tetra-iodophenolphthalein with the bile, Franke (115) proposed that cinchophen causes an acceleration of the cellular diffusion.

The last mentioned observation concerning sodium-tetra-iodophenolphthalein led to the suggestion to administer cinchophen together with this contrast agent so as to obtain a quicker filling of the gallbladder for roentgenographic studies. Pribram (276); and Einhorn and Stewart (94) recommended the use of Biloptin (diiodocinchophen) for this purpose. The preparation was successfully used for some time until toxic reactions forced its withdrawal.

6. SKIN

Oeller (255) noted that patients treated with cinchophen developed an oily skin giving off a peculiar odor that is similar to that possessed by cinchophen, yet no cinchophen was found in the sweat. Neocinchophen is less likely to produce diaphoresis than aspirin.

7. NERVOUS SYSTEM

In the discussion of the mechanism involved in the production of uricosuria, reference was made to the effects of cinchophen upon the autonomic nervous system, associated with this manifestation. Additional data on the autonomic nervous tissue effects were furnished in connection with the presentation of the reactions of cinchophen upon the gastrointestinal tract.

Starkenstein (342) suggested that the inhibition of central glycosuria and the

decrease of the suffocation reaction caused by cinchophen was due to an impaired reaction to central vagus stimulation, while other reactions to cinchophen pointed to a paresis of the sympathetic nerves (Wiechowski and Starkenstein (392); Abl (1)).

Ullmann (368) obtained in the isolated frog's heart perfused with leukotropin a slowing of the heart beat, and a diminished systole. He attributed this effect to a stimulation of the peripheral vagus. Similar changes were produced by Starkenstein, Salus and Wiechowski (343) in rabbits intravenously injected with sodium cinchophen. However, Rotter (300) claimed that cinchophen elicits a paresis of the cardiac nerves with a stoppage of the heart in systole, thereby contradicting Starkenstein (342) and Ullman (368). It is possible that the pH of the perfusion fluid which is shifted to the acid side by cinchophen exerts a definite influence upon the alleged reactivity of the heart to cinchophen. Hydroatophan does not affect the isolated frog's heart; it elicits, however, intense spinal and peripheral stimulation resulting in tetanus and fibrillar contractions, which become manifest upon contact and which are relatively prolonged.

Starkenstein (342) found after large doses of cinchophen given to rabbits a decrease of blood pressure with preservation of splanchnic irritability. Cinchophen apparently produces in rabbits first a stimulation of the vasomotor and vagus centers which is followed by a paresis. Ikeda (183) did not observe any depression of the capillary circulation in the frog's mesentery after the administration of cinchophen. Stake (340) noted that cinchophen accentuates the effect of adrenalin upon the uterus.

Rotter (300) found in frogs weighing 34 grams and given 0.01 gm. of cinchophen general depression followed by a period of increased reflex irritability. Ullmann (368), using leukotropin, also noted a depression and, with the intravenous injection of cinchophen, a miosis. Starkenstein (342) made the same observation as to miosis and obtained this effect also in the atropinized eye.

Through action on the cerebral center of heat regulation causing a lowering of its excitability cinchophen exerts an antipyretic effect which can be elicited even in normal animals (342, 120). Starkenstein (342) did not succeed in lowering the temperature of decerebrated rabbits and of rabbits kept in a warmed box. The antipyretic effect of cinchophen, however, became readily apparent in these warmed animals when they were removed from the warmed box. This observation provided, according to Starkenstein (342), additional evidence as to the central action of cinchophen upon heat control. Dittrich (83) as well as Hesse (171) obtained a severe and sometimes fatal lowering of the temperature of rabbits when cyanamide medication was combined with that of cinchophen. It is important to note that the antipyretic effect of cinchophen was demonstrable in normal animals (rabbits, dogs) only when the drug was given intravenously or subcutaneously, but not when it was administered by mouth. Hyperthermia caused by an injury to the heat center was mitigated by cinchophen (368). Barbour and Lozinsky (11), using dogs made febrile by the injection of typhoid, paratyphoid or B. coli vaccines, found that the ratio of the minimal antipyretic dose to the minimal lethal dose was 1:150 for neocinchophen and 1:163 for cinchophen. Barbour and Winter (13) noted that the combination of magnesium

chloride with cinchophen accentuated the antipyretic effect of the latter in febrile rabbits and dogs. However, the value of this evidence is impaired by the fact that the febrile reactions elicited by vaccines are variable (154), and that the determination of the minimal lethal dose in dogs is made difficult by the great variations in the individual susceptibility of different dogs to cinchophen. Hanzlik, Scott, Weidenthal and Fetterman (155) found neocinchophen a poor antipyretic in rheumatics. Lacquer and Magnus (206) produced in cats a hypnotic state lasting for several days and accompanied by a lowered body temperature.

Zimmer (402) using cinchophen related its antipyretic action to a retention of water in the blood caused by an increased capillary permeability. Barbour and Fisk (9) attributed the antipyretic effect and the dilution of the blood to the accompanying hyperglycemia. Starkenstein and Wicchowski (392) suggested that a part of the antipyretic effect was due to peripheral vasodilatation.

Cinchophen does not act upon the peripheral nerve structure and does not cause any local anesthesia (154). The analgesic effect is central, according to Hanzlik (154). Hesse (171) observed that cinchophen and its derivatives have no analgesic effect in normal tissue as there is no amelioration of the pain sensation in mice given cinchophen when the root of the tail is clamped with a forceps. Tinnitus aurium and other symptoms of the auditory organs occur in 65 per cent of the patients treated with cinchophen, but in only 20 per cent of those receiving neocinchophen (155). Cinchophen causes in rabbits, but not in cats and dogs, a lowering of the respiratory rate through action upon the respiratory center (154). This effect is not influenced by atropin and vagus innervation.

Pollok, Finkelman and Tigay (273) found that the depressant central effect produced by moderate doses of cinchophen in rabbits lowers the convulsant action of minimal convulsant doses of metrazol, but not that of thujone, picrotoxine and alternating current. These investigators suggested that cinchophen perhaps detoxicates metrazol. Such a mechanism may be possible as cinchophen counteracts the muscular action of veratrin by forming a complex with this agent.

8. ANTIPHLOGISTIC ACTION

The antiphlogistic action of cinchophen attracted early clinical attention. The rapid disappearance of the inflammatory signs (redness, swelling, hydrops, pressure pain) of gouty joints was reported by many investigators (382, 342, 91, 196, 343, 154, 163, 233, 368). Similar observations were made in connection with many other inflammatory conditions (arthritis, bronchitis, neuritis, iritis, laryngitis, scleritis, sympathetic ophthalmia) treated with cinchophen (319, 332, 195, 8, 41, 230), where a prompt subsiding of pain, a reduction of passive congestion, and a resorption of inflammatory exudation was noted.

Starkenstein (342) found that cinchophen intravenously or subcutaneously injected, but not when orally given or locally applied, prevents the mustard oil chemosis of the rabbit's eye. Dohrn (85); Lacquer and Magnus (206); and Wicchowski and Starkenstein (392), confirmed this observation, which Lacquer and Magnus (206) could not, however, substantiate in cats. Schikorr (305), on

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chloride with cinchophen accentuated the antipyretic effect of the latter in febrile rabbits and dogs. However, the value of this evidence is impaired by the fact that the febrile reactions elicited by vaccines are variable (154), and that the determination of the minimal lethal dose in dogs is made difficult by the great variations in the individual susceptibility of different dogs to cinchophen. Hanzlik, Scott, Weidenthal and Fetterman (155) found neocinchophen a poor antipyretic in rheumatics. Lacquer and Magnus (206) produced in cats a hypnotic state lasting for several days and accompanied by a lowered body temperature.

Zimmer (402) using cinchophen related its antipyretic action to a retention of water in the blood caused by an increased capillary permeability. Barbour and Fisk (9) attributed the antipyretic effect and the dilution of the blood to the accompanying hyperglycemia. Starkenstein and Wiechowski (392) suggested that a part of the antipyretic effect was due to peripheral vasodilatation.

Cinchophen does not act upon the peripheral nerve structure and does not cause any local anesthesia (154). The analgesic effect is central, according to Hanzlik (154). Hesse (171) observed that cinchophen and its derivatives have no analgesic effect in normal tissue as there is no amelioration of the pain sensation in mice given cinchophen when the root of the tail is clamped with a forceps. Tinnitus aurium and other symptoms of the auditory organs occur in 65 per cent of the patients treated with cinchophen, but in only 20 per cent of those receiving neocinchophen (155). Cinchophen causes in rabbits, but not in cats and dogs, a lowering of the respiratory rate through action upon the respiratory center (154). This effect is not influenced by atropin and vagus innervation.

Pollok, Finkelman and Tigay (273) found that the depressant central effect produced by moderate doses of cinchophen in rabbits lowers the convulsant action of minimal convulsant doses of metrazol, but not that of thujone, picrotoxin and alternating current. These investigators suggested that cinchophen perhaps detoxicates metrazol. Such a mechanism may be possible as cinchophen counteracts the muscular action of veratrin by forming a complex with this agent.

8. ANTIPHLOGISTIC ACTION

The antiphlogistic action of cinchophen attracted early clinical attention. The rapid disappearance of the inflammatory signs (redness, swelling, hydrops, pressure pain) of gouty joints was reported by many investigators (382, 342, 91, 196, 343, 154, 163, 233, 368). Similar observations were made in connection with many other inflammatory conditions (arthritis, bronchitis, neuritis, iritis, laryngitis, scleritis, sympathetic ophthalmia) treated with cinchophen (319, 332, 195, 8, 41, 230), where a prompt subsiding of pain, a reduction of passive congestion, and a resorption of inflammatory exudation was noted.

Starkenstein (342) found that cinchophen intravenously or subcutaneously injected, but not when orally given or locally applied, prevents the mustard oil chemosis of the rabbit's eye. Dohrn (85); Lacquer and Magnus (206); and Wiechowski and Starkenstein (392), confirmed this observation, which Lacquer and Magnus (206) could not, however, substantiate in cats. Schikorr (305), on

decrease of the suffocation reaction caused by cinchophen was due to an impaired reaction to central vagus stimulation, while other reactions to cinchophen pointed to a paresis of the sympathetic nerves (Wiechowski and Starkenstein (392); Abl (1)).

Ullmann (368) obtained in the isolated frog's heart perfused with leukotropin a slowing of the heart beat, and a diminished systole. He attributed this effect to a stimulation of the peripheral vagus. Similar changes were produced by Starkenstein, Salus and Wiechowski (343) in rabbits intravenously injected with sodium cinchophen. However, Rotter (300) claimed that cinchophen elicits a paresis of the cardiac nerves with a stoppage of the heart in systole, thereby contradicting Starkenstein (342) and Ullman (368). It is possible that the pH of the perfusion fluid which is shifted to the acid side by cinchophen exerts a definite influence upon the alleged reactivity of the heart to cinchophen. Hydroatophan does not affect the isolated frog's heart; it elicits, however, intense spinal and peripheral stimulation resulting in tetanus and fibrillar contractions, which become manifest upon contact and which are relatively prolonged.

Starkenstein (342) found after large doses of cinchophen given to rabbits a decrease of blood pressure with preservation of splanchnic irritability. Cinchophen apparently produces in rabbits first a stimulation of the vasomotor and vagus centers which is followed by a paresis. Ikeda (183) did not observe any depression of the capillary circulation in the frog's mesentery after the administration of cinchophen. Stake (340) noted that cinchophen accentuates the effect of adrenalin upon the uterus.

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the other hand, demonstrated a distinct antiphlogistic effect of cinchophen upon the ultraviolet ray dermatitis of rats, even when the drug was orally given. Wiechowski and Starkenstein (392) claimed an antiphlogistic action against experimental cutaneous edema produced in cats by the application of croton oil. Fuerst (120) made similar observations in rabbits with mustard oil blisters of the skin when given cinchophen orally and intramuscularly. Hanzlik and Tainter (156), on the other hand failed to obtain an unequivocal antiphlogistic effect with cinchophen given orally in rabbits on an edema of the head and neck, elicited by the injection of paraphenylenediamine hydrochloride (156). Similarly negative results were reported by Lacquer and Magnus (206) and Gilde-meister and Heubner (127) in rabbits and cats with a pulmonary edema caused by phosgen poisoning.

The mechanism of this antiphlogosis of cinchophen is still a matter of controversy. Starkenstein (342) showed that the effect upon mustard oil chemosis in rabbit's eyes is not influenced by the removal of the superior cervical ganglion, and thus concluded that the reaction is of systemic nature. Investigators of Schering-Kahlbaum (303) brought out the fact that the antiphlogistic effect cannot be transferred from a cinchophen treated animal to another animal through blood transfusion. Fuerst (120) claimed that the antiphlogistic action of cinchophen is causally related to circulatory disturbances set up in the skin, as it was readily obviated when a heating pad was applied to the skin of rabbits with mustard oil blisters. Myers and Killian (246), on the other hand, maintained that the beneficial effect of cinchophen upon arthritic joints is due to an increased blood supply to the affected areas. This concept is supported by the common clinical experience that an increased arterial blood supply to an inflamed region produces a decongestion of this part, resulting in a reduction or disappearance of the edema, a removal of toxic metabolites, a mobilization of the stagnant circulation, an improved oxygenation, and a decrease in pressure pain. Inasmuch as the analgesic effect of cinchophen is seen only in inflamed tissues, it is likely that it is closely related to the antiphlogistic effect of this drug.

Hermann and Zentner (170) suggested that the antiphlogistic effect is the result of an activation of calcium, as cinchophen increases the ultrafiltration of serum calcium. Fuerst (120) as well as Starkenstein (342) pointed out the differences between the inflammation-preventing action of cinchophen and of calcium. While the action of the latter is general, that of the former remains local.

Schikorr (305) proposed that the antiphlogosis might be related to the affinity of oxycinchophen for iron and copper and, therefore, represent a phenomenon of heavy metal catalysis. However, he could not elicit an antiphlogistic effect with synthetic 8-oxycinchophen on actinic dermatitis, while Starkenstein (342) succeeded in eliciting with this chemical such a response in the mustard oil chemosis of the rabbit's eyes.

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9. IMMUNITY

Derick, Hitchcock and Swift (79) reported that neocinchophen prevents nearly completely the formation of circulatory antibodies in serum sickness

caused by the injection of horse serum. Meyer and Mezy (236) stated that cinchophen lessens anaphylactic reactions, whereas Hanzlik (154) noted that cinchophen rather decreases immunity processes than increases them. Gudzent (149) stated that cinchophen, like salicylates and aminopyrine, inhibits allergic reactions of the skin. The inhibitory action of cinchophen thus extends to circulatory as well as fixed or cellular antibodies.

It is obvious from the data presented that many of the most important pharmacologic aspects of cinchophen and its derivatives, especially its mechanism of action, are not well understood and have remained controversial matters.

D. TOXICOLOGICAL ASPECTS

The toxic manifestations elicited by cinchophen and its derivatives are either of chemotoxic or allergotoxic nature. Conforming with the species specific differences in the metabolism of cinchophen there exist definite variations in the degree and type of chemotoxicity of cinchophen for the various species. Reactions of the allergotoxic type have been so far observed in man only, where they are relatively rare. It is possible that such responses, which may involve different organs (skin, liver, vascular system) are not always the result of the development of specific chemoallergens, but may sometimes be related to disturbances in the general antibody status of the organism and due to the injurious action exerted by cinchophen upon the production and activity of fixed circulating immune bodies.

a. *Toxicity and cold blooded animals.* Rotter (300) reported that cinchophen is decidedly more toxic to cold blooded animals than to warm blooded ones. When injected into frogs (*Rana temporaria*) cinchophen paralyzes the central nervous system and the nerves of the heart. The injection of 0.01 gm. of cinchophen into a frog weighing 20 gms. (0.5 gm./kg.) causes death (342). Caffein accentuates the action of cinchophen on the heart, while calcium and atropin do not exert any modifying effect.

b. *Toxicity in warm blooded animals.* *Lethal doses:* Mouse: The injection of 0.005 gm. of cinchophen into a mouse of 25 gms. causes dyspnea, while that of 0.025 gm. (1.0 gm./kg.) produces death (342).

Rat: The lethal dose of cinchophen, given subcutaneously, for rats is 0.5 gm./kg., according to Radwin and Lederer (283) 1.0 gm./kg., according to Reichle (288), and Barbour and Lozinsky (11). The lethal dose of neocinchophen is 20 gms./kg., subcutaneously given (12). The higher the dose of cinchophen, the shorter the survival time. Rats receiving thyroid substance by mouth exhibit a lowered resistance to cinchophen, the minimal lethal dose being reduced thereby from 0.55-0.65 gm./kg. to 0.35-0.40 gm./kg. (122).

Guinea Pig: The lethal dose subcutaneously administered to guinea pigs of cinchophen is 0.9 gm./kg., according to Risi (295).

Rabbit: While the injection of 0.3 gm./kg. of cinchophen does not cause any untoward symptoms in rabbits, that of 0.5 gm./kg. elicits occasional paresis, and that of 0.8 gm./kg., according to Starkenstein, or of 0.95 gm./kg., according to Risi (295), produces death. The oral administration of 1.0 gm./kg. exerts a

lethal effect which is preceded by convulsions and general paresis (342). The lethal oral dose of cinchophen is 1.4 gm./kg. and of neocinchophen 1.0 gm./kg., according to Fuerth and Kuh (121); 0.5 gm./kg. and 0.8 gm./kg. being tolerated without any toxic symptoms. Doses of 0.5 gm./kg. of cinchophen given orally to rabbits proved fatal within 10 days (342).

Cat: The subcutaneous injection of 0.75 gm./kg. of cinchophen into cats causes vomiting (342). 0.2–0.3 gm./kg. orally proved fatal within a few days.

Dog: Risi (295) found that 0.62 gm./kg. of cinchophen, given subcutaneously, is lethal for dogs.

The reliable determination of the single lethal dose of cinchophen administered by mouth is difficult and has not been reliably accomplished, because dogs receiving by this route 0.5 gm./kg. vomit (12), preventing thereby the complete absorption of any higher dose. Barbour and Lozinski (11) stated that 1.25 gm./kg. represents the minimal lethal dose given by mouth, Starkenstein (342) places this dose at 1.5 gm./kg., while Richartz (292) claimed that 0.6 gm./kg. has a lethal effect. Barbour and Lozinsky (11) were unable to obtain a lethal effect with any dose of neocinchophen given to dogs by mouth.

Starkenstein (342) found that 0.5 gm./kg. of cinchophen given for 2 to 3 days orally proved fatal, while Barbour and Lozinsky (11) noted that 3 times 0.33 gm./kg. daily elicited depression and loss of appetite in dogs. When 0.2 gm./kg. of cinchophen was administered by mouth 4 times daily, weakness developed, followed by death on the fourth day. Neocinchophen given in divided doses of up to 9 gms./kg. daily for several days was ineffective in causing death or toxic symptoms, according to Barbour and Lozinsky (11).

Man: No case of acute fatal poisoning with cinchophen or neocinchophen has been reported so far. Willcox (393) contended that the danger of cinchophen poisoning is much enhanced in elderly people. Others maintained that a poor state of nutrition and hepatic disorders predispose to untoward reactions from cinchophen. Sex, apparently, does not play any role in this respect.

1. CHEMOTOXIC REACTIONS

Kidney: Hanzlik et al. (155) reported the occurrence of albuminuria and cylindruria after the administration of cinchophen in high therapeutic doses. Schroeder (310) noted the presence of albuminuria in 5 cases of cinchophen poisoning. Miller and Boots (238), on the other hand, found that the appearance of albuminuria or its disappearance, when preexisting, after cinchophen medication does not follow a definite trend. The available evidence does not support the view that cinchophen exerts a direct cytotoxic effect upon the renal parenchyma. Whenever degenerative changes in the renal tissue were found after toxic doses of cinchophen, these were associated with prolonged depressive states or extensive regressive changes of the liver and thus more likely attributable to the metabolic disturbances set up by these reactions. It is not likely that the three cases of acute hemorrhagic nephritis following cinchophen medication (55, 328, 310) represent chemotoxic effects of the drug. If they have any causal connection with the action of cinchophen, this is possibly of a chemoallergic nature and may be a part of a hepatorenal syndrome.

Seifert (320) reported that cinchophen aggravated a cystitis by the formation of uric acid stones and Weintraud (382) considered for this reason the presence of renal stones a contraindication to the administration of cinchophen. Eimer (93) recommended the administration of alkalis or an intermittent type of cinchophen medication for the prevention of the formation of uric acid concretions. However, Klemperer (196), and Friedeberg (118) never observed the occurrence of renal colics after cinchophen medication.

Respiratory System: The occurrence of dyspnea following the introduction of therapeutic doses of cinchophen was observed by Mendel (233) (diethylenediamine cinchophen) and Zieglwallner (400) (atophanyl). Schilling (306) reported the development of bronchitic symptoms after cinchophen medication. Large doses of cinchophen cause in animals death by respiratory paralysis.

Auditory System: The development of tinnitus aurium and vertigo after cinchophen medication was previously mentioned as a part of the symptom complex of cinchophenism (154, 35, 336). This toxic syndrome occurs less often following the administration of neocinchophen.

The alleged production of unilateral deafness by neocinchophen (213) cannot be taken seriously as this particular patient suffered from auditory disturbances before the cinchophen therapy was instituted.

Nervous System: Rawls (285) mentioned that the therapeutic use of cinchophen causes not infrequently disturbances of the nervous system, manifesting themselves in emotional instability (22%), mental depression (6%), insomnia (4%), headache, dizziness and fatigue. The incidence of such reactions is reduced, according to Rawls, by the simultaneous medication of vitamin K (emotional instability (2.75%), mental depression (1.25%), insomnia (2.5%)).

Dogs and monkeys receiving orally toxic doses of cinchophen become progressively depressed and somnolent.

Gastrointestinal System: The occurrence of untoward gastrointestinal reactions following the use of cinchophen was reported soon after the introduction of this drug into medical therapy. The symptoms observed are abdominal distress, nausea, anorexia, vomiting, pyrosis, diarrhea and constipation. Loss of appetite and impaired nutrition are not infrequent sequelae of these reactions, which are evidently the result of the prolongation of the secretory activity of the gastric mucosa elicited by cinchophen and which may necessitate the discontinuation of this medication. They may precede, accompany or follow the appearance of toxic manifestations from other organs, especially the liver. Westfall (389) reported that gastrointestinal complaints were noted in 98 of 1598 cases treated with cinchophen. This incidence seems to agree with the observations made on this subject by the majority of investigators. Rawls, on the other hand, gives a much higher frequency of such untoward responses: Nausea in 36 per cent, vomiting in 4 per cent, diarrhea in 2 per cent, abdominal pain in 22 per cent, pyrosis in 10 per cent, and constipation in 3 per cent.

There exists very little evidence suggesting that such reactions may lead to the development of peptic ulcers in the stomach and duodenum of man. So far only 2 cases are placed on record in which a causal relationship between peptic ulcer of the stomach and preceding therapy with cinchophen was considered a

possibility (287, 30). The patient in Rhea's case (287) was a heavy drinker who received cinchophen because of arthritis and who showed at autopsy an ulcer of the lesser curvature. In view of the fact that chronic alcoholism is often associated with chronic gastritis, it is more likely that this factor played the deciding role in the production of the ulcer. In the second case reported by Bloch and Rosenberg (30) various vaccines were injected into the patient, an old woman with polyarthritis, before cinchophen was given. One ulcer was found at the junction of the esophagus with the stomach, while a second one was situated somewhat lower in the stomach. Both locations are not typical for cinchophen ulcers which are located at the pylorus, at the lesser curvature and in the postpyloric part of the duodenum. Peptic ulcers of the stomach are moreover so frequent (5-10 per cent) in the general population that Bloch and Rosenberg conceded the probability of coincidence.

Experimental studies on dogs, cats and chickens, have shown, on the other hand, that chronic cinchophen medication readily elicits in these species peptic ulcers in the stomach and duodenum (61, 62, 372, 153, 324, 316, 289, 341, 327, 9, 396, 291, 252, 357, 74, 59, 182). Attempts to elicit similar ulcerative lesions in the stomach or duodenum of rabbits, guinea pigs, and monkeys, however, failed, indicating that a species specific response is involved (324, 316, 182).

The ulcerative gastric lesions were elicited by giving dogs 5, 10 and 25 times the daily therapeutic dose employed for man, which is 0.022 gm./kg. The cinchophen suspended in cotton seed oil, gum acacia or starch solution was administered by stomach tube. Dogs frequently vomited after such medication, and lost appetite and weight. During the first week on such a management, they grew weak and lay in their cages in a sort of doubled up position.

Peptic ulcers develop within 10 to 60 days after the start of the ingestion of cinchophen, depending upon the susceptibility of the individual dog and the dose of cinchophen administered, and may affect 100 per cent of the dogs subjected to the treatment. While some of the dogs may die from the toxic effects of cinchophen without developing ulcers during the acute stage of the experiment, those which survive often show a return of their appetite, may even gain in weight, and may develop ulcers before any nutritional disturbances become manifest (341). With prolonged medication and ulcer formation, a generally weakened condition appears, associated with tarry diarrhea at times. The total amount needed for the production of gastroduodenal ulcers varied from 1.23 gm./kg. to 27.06 gm./kg., the average being 5.16 gm./kg. (372). Inasmuch as cinchophen administered by other routes (subcutaneous, intravenous, rectal, intestinal fistula) elicited the same effects (74, 324, 153, 289, 9, 341), it is obvious that the ulcerative lesions are not the result of a local action of cinchophen upon the gastric mucosa, but are related to the prolongation of the gastric secretion occurring under the influence of cinchophen. It is for this reason that peptic ulcers develop more rapidly in Heidenhain's pouches, which do not come in contact with orally given cinchophen, than in the rest of the gastric mucosa, because the mucosa in the pouches is continuously exposed to the stagnating acid gastric secretion (341), which, however, does not show any hyperacidity.

The first changes occurring in the gastric mucosa are of the acute inflammatory nature and appear about 24 hours after the ingestion of cinchophen or 6 to 10 hours after its subcutaneous injection (153, 327). This primary stage of acute gastritis lasts from one week to ten days (327, 341) and is characterized by mucosal edema, infiltration with lymphocytes and plasma cells, small hemorrhagic erosions and narrow fistulous ducts extending into the mucosa and filled with leucocytes. The fistulas originate from the destruction of the mucosa in the depth of the crypts.

During the second stage there occurs a gradual return of the gastric mucosa to a normal condition in most parts of the stomach with the exception of those portions where a progressive ulcerative development of some of the erosions into deep chronic ulcers takes place. These have necrotic and inflamed bases and indurated walls and resemble, in most respects, the peptic ulcers found in man. Sometimes the ulcers extend into the muscularis and may even perforate with erosion of and hemorrhage from vessels located in the bases.

The ulcers persist as long as cinchophen is given. However, a few days after the cessation of cinchophen medication, the bases become clean and fill in with granulation tissue. Complete healing usually is accomplished within 2 to 3 weeks (357).

The ulcers are usually located in the prepyloric region or directly on the pyloric ring (89.3 per cent). Others are situated at the lesser curvature or in the postpyloric part of the duodenum (7.1 per cent). They are rarely found on the anterior wall or the greater curvature. Occasionally, also, jejunal and ileal ulcers are seen. An association of duodenal ulcers with gastric ones occurs in about 10 per cent of the cases. Multiplicity of ulcers exists in 32 to 35 per cent of the cases (341).

These investigators found that the dogs receiving daily 2 gms. of cinchophen in a coarse diet develop more quickly gastric ulcers than dogs fed a soft diet. When 30 gms. of gastric mucin was given twice daily or when pectin was administered with the cinchophen, the incidence of ulcer formation was considerably reduced (by 50 per cent and 89 per cent, respectively) (289, 396, 291). The cutting of the pyloric sphincter muscle (Rammstedt operation), obviating thereby the development of a pylorospasm, generally elicited by cinchophen, also reduced the incidence of ulcer formation by about 50 per cent by permitting the free drainage of the injurious gastric secretion into the intestine (74). The pylorospasm was also abolished by atropine, but not by the bilateral sectioning of the vagi in the neck. Cholecystogastrostomy, on the other hand, had no effect upon the frequency of gastric ulcers (357).

Observations made by Davis, Bradley, Bachrach, and Ivy (74) on the bilirubin clearance and the serum phosphatase of dogs with cinchophen ulcers revealed that there was no evidence of an associated functional liver damage and thus no demonstrable causal connection between a potential primary liver injury and the ulcer formation of the stomach. The histologic examination of the liver of such ulcer dogs showed the presence of normal conditions (341, 357). Dogs with cinchophen ulcers studied by Hueper (182) exhibited mild to moderate

interstitial edema and scattered degenerative changes in the liver. Reymont (291) stated that cinchophen may cause a disturbance of liver function without simultaneously eliciting peptic ulcers.

Cinchophen is excreted into the gastric juice of dogs at a maximum rate of 1.5 mgm. per hour.

Cheney (59) obtained in 3 week old chicks fed an antigizzard deficient diet and cinchophen, penetrating, perforating and, often multiple, ulcers of the stomach within 24 to 48 hours after the start of the experiment. A protective diet contained high amounts of carbohydrates. Cheney (59) assumed that cinchophen interferes with the proper functioning of the liver and thereby with the adequate delivery of protective substances from the liver to the gastric mucosa.

Hematopoietic System: Mention has already been made of the fact that excessive doses of cinchophen fed to rats kept on a vitamin K low diet (182) or administered to man over prolonged periods of time (285) causes a lowering of the prothrombin content of the blood.

Kracke (203) and Plum (270) mentioned in their reviews on agranulocytosis a doubtful case of this condition allegedly elicited by cinchophen. Rawls (285, 286) reported recently a new case of agranulocytosis in a patient who received cinchophen and 1,4 naphthoquinone, but attributed the hematic condition to the action of the vitamin K preparation. Shapiro and Lehman (321) recorded a fatal case of agranulocytosis after the ingestion of cinchophen and Beckermann (23) observed in 2 cases with severe liver injury following cinchophen medication a moderate degree of leukopenia. If these leukopenic reactions are actually due to cinchophen, they belong into the group of allergic drug reactions such as those seen after aminopyrine, sulfonamides, thioureas, and other organic chemicals.

2. CHEMOALLERGIC REACTIONS

Skin: Among the various chemoallergic manifestations seen in man after the administration of cinchophen or one of its derivatives, those of the skin attracted early attention and are among the more frequent chemoallergic reactions to this drug (159). They may appear a few hours after the apparently first contact of the affected person with the drug or they may develop following a prolonged medication. There seems to exist, therefore, an idiopathic type of cutaneous hypersensitivity and an acquired one, unless the supposition of Sugg (353) is correct that even in the "idiopathic" cases a previous contact with the chemical occurred, which was obscured by the fact that cinchophen is a constituent of numerous patent medicines, often taken without the knowledge of their cinchophen content.

These cutaneous reactions take the form usually of itching, purpuric, urticarial or scarlatiniform eruptions or of exfoliative erythroderma and are sometimes associated with angioneurotic edema. They are often accompanied by chemotoxic and allergotoxic manifestations of other organs (fever, headache, chills, depression, syncope, aphthous ulcers of mucous membranes, nausea, vomiting,

anorexia, jaundice, swelling of the joints) and are observed after an exposure to cinchophen, neocinchophen, atophanyl and oxyliodide (353, 314). A total of 65 cases of dermoallergic reactions to cinchophen have been reported (168, 77, 231, 181, 40, 194, 366, 310, 398, 35, 306, 356, 105, 237, 93, 325, 314, 211, 309, 389, 277, 288, 73, 229, 353, 285).

The absolute and relative incidence of such complications is not high considering that Westfall (389) found only 13 instances of cutaneous reactions among 2,467 cases treated with cinchophen, of which 1,589 cases were analyzed. Snyder (334) remarked that he saw urticaria in only a few of 2,500 patients receiving cinchophen. Rawls (285), on the other hand, asserted that 20 per cent of 200 cases treated with cinchophen and 4.5 per cent of all cases receiving cinchophen plus bile salts or bile salts and vitamin K developed cutaneous reactions. The observations made by Rawls (285), however, differ strongly from the findings recorded by many clinicians who used cinchophen extensively in their practice.

In several instances cutaneous reactions recurred whenever the drug was taken after varying intervals of time (273, 310, 318, 77, 40, 194, 231, 268). Skin tests for the demonstration of chemoallergy to cinchophen were positive in 2 cases studied by Fink (105), but were negative in cases investigated by Miller (237), and Short and Bauer (325).

Rawls, Gruskin, Ressa and Gordon (286) mixed 1 cc., 2cc., and 3 cc., of a 5 per cent sodium cinchophen solution, respectively, with 5 cc. of serum and kept these mixtures in the refrigerator for 4 days. When 50 patients, who had daily received 0.5 gm. of sodium cinchophen for 4 weeks, were injected intradermally with 0.05 cc. of these mixtures, 74 per cent of those showing positive skin reactions exhibited toxic symptoms, including cutaneous reactions, while 71 per cent of those with normal liver function tests revealed a negative skin test. Suggs (353) listed in a group of 10 cases with skin reactions 2 fatal cases. It must be emphasized, however, that these fatalities were not the result of the cutaneous complications, but were caused by associated liver injuries (356). Hepatic reactions were present in 6 of the 41 cases of cutaneous cinchophen allergies reviewed by Short and Bauer (325).

Of the 26 cases collected by Short and Bauer (325) with data available on sex, 12 were males and 14 females. The age varied from 13 to 74 years. The dose after which the eruptions appeared ranged from 1 gm. of cinchophen taken within 6 hours to 115 gms. used within 114 days.

It may be mentioned that Rawls (285) observed in 5 out of 9 cases with cinchophen urticaria and Hench (164) in 16 with this condition a striking relief of the arthritic pains. This improvement lasted for from 3 weeks to 5 months depending in general upon the duration and severity of the urticarial reaction.

The appearance of cutaneous manifestations as evidence of a hypersensitivity to cinchophen represents an absolute indication for the immediate cessation of any further administration of the drug. The cutaneous lesions may be treated with an antipruritic lotion, with intramuscular injections of calcium gluconate and with oral administrations of tricalcium phosphate (237). Short and Bauer

(325) advised that prophylactically intravenous injections of dextrose solution be given for a period of about a week so as to counteract any simultaneous liver damage.

Cardiovascular System: Episodes of circulatory failure characterized by the appearance of tachycardia, palpitations, low blood pressure, apprehension, precordial tension, pallor, chill, flushes, restlessness, nausea, and syncope were seen in a number of cases treated with cinchophen (318, 14, 356, 314, 99). In several of them they were associated with allergic cutaneous reactions (310, 277, 123). Skin tests for hypersensitivity to cinchophen were negative in the cases reported by Quick (278) and Barron (14).

Liver

The occurrence of jaundice associated with acute or chronic degenerative and necrotizing changes in the liver appears to be the most frequent and, at the same time, the most dangerous complication ascribed to cinchophen and its various derivatives. These hepatic reactions assume, in an appreciable percentage of the cases, the character of an acute or subacute yellow atrophy of the liver or of a cirrhosis of this organ, which have, not infrequently, a fatal outcome. It is for this reason that such untoward responses to cinchophen have attracted considerable attention during the last twenty years (261, 46, 386, 73, 384, 325, 259). In 1936, Palmer and Woodall (259) collected 192 fatal and nonfatal cases of jaundice following the administration of cinchophen or one of its derivatives. Since that time some 40 additional cases have been placed on record, making a total of approximately 230 cases (398, 48, 128, 393, 388, 198, 98, 174, 193, 315, 328, 162, 284, 78, 262, 280, 381, 219, 218, 356, 72, 243, 41, 117, 3, 288, 215, 105, 361, 207, 370, 261, 378, 299, 322, 93, 32, 97, 242, 64, 175, 123, 216, 386, 26, 18, 22, 151, 226, 287, 261, 73, 384, 394, 65, 383, 339, 333, 229, 81, 377, 50, 146, 51, 265, 184, 337, 264, 187, 355, 241, 224, 140, 180, 67, 178, 116, 30, 204, 294, 380, 72, 228, 260, 326, 23, 70, 334, 335, 389, 201, 126, 202, 53, 82, 103, 225, 15).

In a considerable number of these cases premonitory symptoms of cinchophen toxicity, such as bloating, nausea, vomiting, gastric distress, rashes and itching of skin, loss of appetite, fatigue and headache, preceded the onset of jaundice. In many others, however, jaundice was the first sign of an untoward reaction to cinchophen.

Weir and Comfort (384) have divided the cases of cinchophen hepatotoxicosis into five groups according to the degree of severity. In the first group, representing the mildest type of toxic reaction, there are cases in which the liver is enlarged and cirrhotic, the van den Bergh test and the bilirubin content of the serum are normal, but the bromosulphthalein test indicates an impaired liver function. In the second group, comprising somewhat more severe degrees of hepatic injury, mild jaundice exists in conjunction with a normal sized, but nodular, or an enlarged liver. The bilirubin content of these cases varies between 4.6 to 5.8 mg. per 100 cc., and the bromosulphthalein test is positive. The third group contains the cases with moderately severe jaundice. The serum bilirubin level stands around 10 to 15 mg. per 100 cc., and the bromosulphthalein test is positive. Tyrosine may be present in the urine. In the fourth group jaundice is

severe, the bilirubin content of the serum ranges around 15 to 25 mg. per 100 cc. The bromosulphthalein test is strongly positive. The stool is clay colored; the liver is not palpable. There develops considerable loss of weight, strength and appetite. Pain in the abdomen is often complained of and ascites is occasionally present. The patient is drowsy and often feverish. Icterus index and van den Bergh tests are positive. A leucocytosis is frequently found. The urine contains bilirubin, urobilinogen and, sometimes, tyrosin. The prognosis of these cases is grave and recovery extends over many months. An impaired liver function is demonstrable long after actual jaundice has disappeared. The fifth group is composed of the fatal cases which exhibit a symptomatology similar to that seen in the previous group. The bilirubin content of the serum often reaches high figures. Stupor, delirium and hyperpyrexia usually are present during the terminal stage.

In some cases jaundice regressed for some time, but later recurred and progressed to a fatal outcome. The symptomatology of cinchophen hepatotoxicosis does not fundamentally differ from that seen in liver injuries caused by other poisons or developing on an obscure basis. It is noteworthy that cinchophen injury of the liver may assume the character of a simple catarrhal jaundice, as well as that of a fulminating acute yellow atrophy of the liver, or of a chronic cirrhosis of this organ with transitions from one stage into another. In this respect it resembles the hepatic manifestations seen in infectious hepatitis, recently described by Lucké (221). The prognosis of cinchophen hepatitis is always serious and guarded even after apparent recovery because of the possibility of a subsequently developing cirrhosis (393). The prognosis is especially grave during the acute stage in the presence of a small liver, ascites, and cinchophen medication continued after the appearance of jaundice. The bromosulphthalein test is of little prognostic value, but helps to gauge the degree of liver injury, and thus the degree of functional recovery after the jaundice has cleared up.

In 50 fatal cases 52 per cent died within 2 weeks after the appearance of jaundice, 74 per cent within 4 weeks, and 86 per cent within 6 weeks. In 40 of 56 nonfatal cases recovery occurred in 10 per cent within 2 weeks, in 5 per cent within 4 weeks, in 57.5 per cent within 6 weeks, and in 85 per cent within 8 weeks. Ascites developed in 11 cases after 3 weeks of jaundice, while only 2 of 13 cases with ascites recovered. Edema without ascites was present in 10 cases and with ascites in 9 cases (384).

The autopsy reveals changes such as are commonly seen in acute yellow atrophy and cirrhosis of the liver. The weight of the livers ranged from 450 gms. (3) to 1,950 gms. (288). In only 12 of 43 cases did the weight of the livers surpass 1,000 gms. (259). The histological changes found in the livers with cinchophen hepatitis correspond to those observed in acute yellow atrophy or toxic cirrhosis of the liver. The kidney often reveals degenerative changes of the tubular epithelium. It is probable, however, that these lesions are not the result of a specific toxic action of cinchophen, but are related to the metabolic disturbances brought about by the liver injury. Autopsy reports were available in 70 of the 101 fatalities recorded.

The sporadic and capricious occurrence of cinchophen hepatotoxicosis has greatly

complicated the task of elucidating the etiologic factors involved in the production of these therapeutic accidents. Despite a careful analysis of the clinical data and a great deal of experimental work, a satisfactory solution of this problem has not yet been found.

The following primary conditions existed in 172 cases treated with chlophane and subsequently developed jaundice: pyelitis, usually of staphylococcal origin, rheumatism, arthritis, lumbago, neuritis, rheumatoid pain, sinusitis, tonsillitis, traumatic pain, pain after extraction of tooth, epigastric pain, pain of indolent origin, grip, cold, exsams, lymphangitis, pleurisy, gonorrhea, and cholecystitis. Their incidence is listed in Table 3.

Rheumatic and other infections, as well as antenatal conditions of various etiology, were present in the majority of cases, while gonorrhea furnished only a small number despite the fact that this disease represents the earliest and most promi-

TABLE 3
Primary Disease in Circulating Eosinophils

ILLNESS	CASES	ILLNESS	CASES
Rheumatism.....	40	Cold.....	3
Rheumatic Arthritis.....	2	Infections.....	4
Acute Arthritis.....	2	Gonorrhea.....	0
Chronic Arthritis.....	63	Miscellaneous conditions.....	4
Nervous.....	11	Cholelithiasis.....	0
Miscellaneous Pains.....	12		

TABLE 4
Age Distribution of Female Members of Women's

Date	Amount					
	1911	1912	1913	1914	1915	1916
January	100.00	100.00	100.00	100.00	100.00	100.00
February	100.00	100.00	100.00	100.00	100.00	100.00

next indication for cinchophen therapy (164). There was, on the other hand, a relatively high incidence of cinchophen hepatitis in individuals receiving this drug for cholecystographic purposes. The incidence appears to be excessive as cinchophen preparations were used for only a very limited time and evidently in only a restricted number of patients for the purpose of the gallbladder.

It has been minimally susceptible her exposure
increases with age (225) age dependent morbidity and
mortality after diagnosis represent

The data indicate that the rate for all age groups calculated by Palmer and Woolf. The earliest age group seen discrepancy may be due to the

ence of infectious diseases in these individuals contributing to the lethal outcome of the hepatic complication.

The peak of incidence of cinchophen hepatitis occurs in the fifth to seventh decade, i.e., at a time when arthritic conditions are particularly frequent. As the incidence rate decreases with advancing age, it is not likely that age as such has any influence upon the susceptibility to cinchophen hepatitis. It is possible, on the other hand, that the chances for an acquired sensitivity to cinchophen are enhanced with age.

The sex was known in 190 cases; 75 were males and 115 females. While it is doubtful whether or not this sex distribution has any significance, the expectancy toward recovery is greater in males than in females. Bryeo (46) found that 30 per cent of 73 males died, while 54 per cent of 111 females died.

In the great majority of cases cinchophen was taken by mouth. Parenteral administration was practiced in only 22 cases, in five of which the drug also was given orally. This distribution apparently reflects the types of clinical use of

TABLE 5
Distribution of Cinchophen Preparations

PREPARATION	CASES	PREPARATION	CASES
Cinchophen	119	Weldona	2
Neocinchophen	10	Renton's Hydracino	9
Atophanyl	2	Cass Rheumatism Remedy	2
Oxydodido	13	Harvell's Rheumatism Cure	1
Atochinol	1	Van Ard's Rheumatism Cure	2
Atoquinol	1	Cinca-Vess	1
Biloptin	6	Gorum Cachets	2
Quinophan	2	Radiophan	1
Faristan	6	Areanol	5
Quinofan	1	Iriphan.	1
Guansin	2		

cinchophen preparations, and does not indicate that the way of introduction of cinchophen plays any definite role in determining the development of toxic hepatic reactions. It has been noted, however, that the onset of hepatic symptoms is hastened after the intravenous injection of cinchophen (193, 174, 317, 328, 162, 288).

The route of medication (oral, parenteral) showed the following relation to the outcome of hepatic accidents: Parenteral administration (intravenous and intramuscular): 13 cases with 4 deaths (25%) and 9 recoveries; oral administration: 202 cases with 98 deaths and 104 recoveries (50%). It is not likely that the difference in mortality rates between oral and parenteral medication reflects an actual difference in the toxicity of cinchophen when given by different routes. It is more probable that the higher amounts of cinchophen when given by mouth, and the longer duration over which cinchophen is given orally in general, accounts for the discrepancy.

The frequency with which cinchophen and its various derivatives and preparations contributed to these complications is seen from Table 5.

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The following primary conditions existed in 179 cases treated with cinchophen and subsequently developed jaundice: polyarthritis, usually of rheumatic origin, rheumatism, arthritis, lumbago, neuritis, rheumatoid pain, sinusitis, toothache, traumatic pain, pain after extraction of tooth, epigastric pain, pain of indefinite origin, grip, cold, eczema, lymphangitis, pleurisy, gout, and cholecystitis. Their incidence is listed in Table 3.

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DIAGNOSIS	CASES	DIAGNOSIS	CASES
Rheumatism.....	40	Cold.....	5
Rheumatic Arthritis.....	8	Infections.....	4
Acute Arthritis.....	6	Gout.....	8
Chronic Arthritis.....	65	Miscellaneous conditions.....	4
Neuritis.....	17	Cholecystography.....	6
Miscellaneous Pains.....	16		

TABLE 4
Age Distribution of Hepatic Morbidity of Mortality

	AGE: YEARS							TOTAL CASES
	10-20	21-30	31-40	41-50	51-60	61-70	71-80	
Morbidity.....	4	18	28	41	50	35	4	180
Mortality.....	4	5	12	22	21	21	2	87

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It has been maintained that the susceptibility to cinchophen hepatitis increases with age (225, 46, 393). The age distribution of hepatic morbidity and mortality after cinchophen medication is represented in Table 4.

The data indicate that the mortality rate is about 50 per cent of the morbidity rate for all age groups. This figure agrees with the rate of 46.8 per cent calculated by Palmer and Woodall (259, 260) for a series of 191 cases with 88 deaths. The earliest age group seems to represent an exception to this rule, but this discrepancy may be due to the small number of cases in this group and to the pres-

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Atoquinol	1	Cinsa-Vess	1
Biloptin	6	Gorum Cachets	2
Quinophan	2	Radiophan	1
Farastan	6	Arcanol	5
Quinofan	1	Iriphan	1
Guaiasin	2		

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In the majority of cases of hepatitis cinchophen had been administered either continuously or in intervals over periods of weeks or months. The shortest interval between the medication of cinchophen and the onset of jaundice was 2 days. In some cases jaundice made its appearance some weeks or months after the cessation of cinchophen medication (219, 30, 393, 151). This delayed type of reaction indicates that, occasionally, a slowly progressive hepatotoxic response is elicited which continues after the initial injuring agent has ceased to act.

The available clinical evidence thus shows that, while some persons possess an abnormal susceptibility to therapeutic doses of cinchophen which is expressed in various chemotoxic and allergic reactions of various organs including the liver, the great majority of patients receiving this drug enjoy a relative immunity, as they are able to take even large doses of cinchophen over long periods with impunity.

Various hypotheses have been advanced in attempts to explain the mechanism of hepatotoxic action of cinchophen. An opinion widely held attributes these hepatic injuries to a direct toxic action of the drug upon the liver cells (225). Eimer (93) claimed that the toxic effect results from a gradual accumulation of cinchophen and its metabolites in the body, finally reaching a level at which these chemicals become injurious to the liver cells. A similar theory was proposed by Straub (352) who contended that a continued administration of cinchophen does not permit a recovery of the liver cells from the damaging action of the drug, thereby ultimately causing extensive degenerative and necrotizing changes in the liver. The process of hepatic regressive alterations, according to Straub (352), may proceed for some time before causing a manifest jaundice, which signalizes a more massive breakdown of liver cells. Sherwood and Sherwood (322) suggested that the hepatotoxic action of cinchophen is the result of an abnormal metabolic decomposition of the drug causing the development of nitrobenzene from the quinoline nucleus, which in turn is not adequately excreted by the kidneys. Rabinowitz (280), Willcox (393), and Weis (386), on the other hand, held that the benzene-nucleus in the cinchophen molecule is to blame for the liver injury, as it exerts a similar effect in arsenobenzol and trinitrotoluol. In a recent report on cinchophen by the Council on Pharmacy and Chemistry (1941) the statement is made that cinchophen hepatitis is of chemotoxic and not of allergic origin. In support of this contention, it was maintained that similar changes had been produced by cinchophen in experimental animals.

Commenting on the theories of Sherwood and Sherwood, and of Rabinowitz, it may be noted that the alleged conversion of the quinoline nucleus into nitrobenzene or benzene is a purely speculative supposition (278) and that benzene as such does not, as a rule, exert a hepatotoxic effect, leading to acute yellow atrophy or cirrhosis of the liver. If the assumption that cinchophen actually injures the liver cells is correct, there should be demonstrable a direct and consistent relation between the dose of cinchophen administered and the total consumption of the drug, on the one side, and the occurrence and frequency of cinchophen hepatitis on the other. Cinchophen thus should follow the generally recognized laws of toxicity as they are exemplified in regard to a specific toxic action upon the liver by numerous chemicals (phosphorus, chlorinated aliphatic

hydrocarbons (chloroform, carbon tetrachloride, trichlorethane, ethylene dichloride, tetrachlorethylene), trinitrotoluol, trinitrophenol, dinitrophenol, dinitrobenzol, chlorinated naphthalenes, and toxins of certain mushrooms and infectious agents (Weil's disease, yellow fever) (66, 280, 393).

In the preceding discussion concerning the relationship between the dose received and the development of jaundice, it was brought out that there do not exist any fixed connections between these two factors, as the dose varied over a wide range and, in many instances, was definitely far below any recognized toxic dose for this chemical. Thus cinchophen does not obey the accepted rules existing between quantity of a chemical administered and toxic effect elicited.

TABLE 6

Annual Distribution of Cinchophen Hepatitis and Production of Cinchophen

YEAR	NUMBER OF HEPATITIS CASES PRESENT SERIES	ALL TOXIC REACTIONS (BRYCE)			CINCHOPHEN PRODUCTION	NEOCINCHOPHEN PRODUCTION
		U. S. A.	World			
			Total	Fatal	In U. S. A.	
					lbs.	lbs.
1923	1	0	1	0	32,710	2,725
1924	0	3	3	0	56,003	4,667
1925	1	1	1	1	60,722	5,060
1926	12	0	13	4	79,632	6,636
1927	15	0	11	3	84,212	7,017
1928	10	0	7	5	94,330	7,860
1929	8	4	8	3	99,538	8,294
1930	12	9	21	4	93,765	8,354
1931	33	16	26	19	80,000*	6,667
1932	39	34	37	18	60,000*	5,000*
1933	39	21	30	12	47,000*	3,911
1934	25	28	31	14	40,000*	3,300*
1935	12	3	7	3	26,000*	2,166
1936	13		24	11		

* Estimated by Bryce (46).

The analysis of the case reports on hepatic injuries shows that the incidence of such accidents has greatly varied in different years. It is reasonable to assume that there should exist a demonstrable relation between the annual incidence of hepatotoxic reactions and the annual consumption of cinchophen, as given by the U. S. Census of Dyes and Other Synthetic Chemicals (46), if cinchophen exerts a direct toxic effect upon the liver cells (Table 6).

The production figure for neocinchophen, according to the same source, stood at 6,946 lbs. in the year 1940, and at 7,515 lbs. in 1942. It is noteworthy in this connection that, on the basis of the available sales figures, the therapeutic use of cinchophen has definitely increased since 1940. The incidence of cinchophen hepatitis, on the other hand, as reflected by the published reports, has almost reached the vanishing point during recent years. While there were 13 cases

placed on record in 1936, 3 were reported in 1937, 1 in 1938, 6 in 1940, and 1 in 1942. It is likely that because of the present world conditions some of the cases may have escaped notice and that others were never reported. Nevertheless, it is believed that the available figures reflect an actual trend and that the incidence of cinchophen hepatitis has definitely decreased.

The available evidence indicates that there does not exist a parallelism between the number of reported cases of this disease and the amount of cinchophen and neocinchophen produced in the United States. It cannot be maintained that the decrease of cinchophen hepatitis reported is due to a reduction of the unfavorable publicity which cinchophen has received during the last ten years, as the J.A.M.A. has not only published several papers on this subject during this period, but has contained also reports from the Council of Pharmacy and Chemistry (69), several queries and letters dealing with the hazards connected with the use of this drug. The prediction of Hench (161) made in 1932, that there would be an increase of the yearly rate of 6 deaths from cinchophen throughout the world, when the condition would receive wider recognition, has not come true.

It is apparent that an unusually large proportion of the recorded cases originated in the United States, although it is likely that the drug has enjoyed a more general and indiscriminate use on the European continent. However, not only the regional distribution of cinchophen hepatitis appears to be erratic, but also that reported from different hospitals and by different investigators. Whether such discrepancies indicate variations in the therapeutic use and in the supervision of the patients remains an open question.

Eaton (88) stated that, between 1933 and 1935, he and his associates administered to arthritic patients in the hospital and in private practice 6,000 ampoules of phenylcinchoninic acid in methenamine, and 63,000 capsules of cinchophen in methenamine and 33,000 capsules of neocinchophen in methenamine without a single instance of deleterious complication. Motzfeldt (243) noted in 1929 that 2,000 gms. of cinchophen were dispensed in 1927 in the Norwegian Hospital with only one case of jaundice, while none was observed in preceding years. Fleischmann (107) mentioned that in the Moabit Hospital in Berlin 8,000 gms. of cinchophen were prescribed in 1931 without a single therapeutic accident. Klemperer (196) was quoted by Fleischmann to the effect that 20 kg. of this drug were used within 2 years in the same hospital without any apparent ill effects. Snyder, Traeger, Zoll and Lust (335) administered cinchophen to 2,560 patients within 10 years and observed only one case of mild jaundice and a few cases of urticaria. Curschmann (70) prescribed cinchophen for over 20 years and observed only one case of mild liver injury. Schittenhelm (307) made similar favorable observations at the Medical Clinic at Munich.

Less favorable are the observations made at the Hertzler Clinic, according to the report of Westfall (389). Data were compiled from 1,589 cases, out of a total of 2,467 cases, treated with cinchophen (886 cases from 25 to 100 tablets, 195 cases from 250-500 tablets, 47 cases from 500 to 1000 tablets, 11 cases from 1000 to 1500 tablets). Several patients took the drug daily for 3 to 4 years.

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1930	12	9	21	4	93,765	8,354
1931	33	16	26	19	80,000*	6,667
1932	39	34	37	18	60,000*	5,000*
1933	39	21	30	12	47,000*	3,911
1934	25	28	31	14	40,000*	3,300*
1935	12	3	7	3	26,000*	2,166
1936	13		24	11		

* Estimated by Bryce (46).

The analysis of the case reports on hepatic injuries shows that the incidence of such accidents has greatly varied in different years. It is reasonable to assume that there should exist a demonstrable relation between the annual incidence of hepatotoxic reactions and the annual consumption of cinchophen, as given by the U. S. Census of Dyes and Other Synthetic Chemicals (46), if cinchophen exerts a direct toxic effect upon the liver cells (Table 6).

The production figure for neocinchophen, according to the same source, stood at 6,946 lbs. in the year 1940, and at 7,515 lbs. in 1942. It is noteworthy in this connection that, on the basis of the available sales figures, the therapeutic use of cinchophen has definitely increased since 1940. The incidence of cinchophen hepatitis, on the other hand, as reflected by the published reports, has almost reached the vanishing point during recent years. While there were 13 cases

placed on record in 1936, 3 were reported in 1937, 1 in 1938, 6 in 1940, and 1 in 1942. It is likely that because of the present world conditions some of the cases may have escaped notice and that others were never reported. Nevertheless, it is believed that the available figures reflect an actual trend and that the incidence of cinchophen hepatitis has definitely decreased.

The available evidence indicates that there does not exist a parallelism between the number of reported cases of this disease and the amount of cinchophen and neocinchophen produced in the United States. It cannot be maintained that the decrease of cinchophen hepatitis reported is due to a reduction of the unfavorable publicity which cinchophen has received during the last ten years, as the J.A.M.A. has not only published several papers on this subject during this period, but has contained also reports from the Council of Pharmacy and Chemistry (69), several queries and letters dealing with the hazards connected with the use of this drug. The prediction of Hench (164) made in 1932, that there would be an increase of the yearly rate of 6 deaths from cinchophen throughout the world, when the condition would receive wider recognition, has not come true.

It is apparent that an unusually large proportion of the recorded cases originated in the United States, although it is likely that the drug has enjoyed a more general and indiscriminate use on the European continent. However, not only the regional distribution of cinchophen hepatitis appears to be erratic, but also that reported from different hospitals and by different investigators. Whether such discrepancies indicate variations in the therapeutic use and in the supervision of the patients remains an open question.

Eaton (88) stated that, between 1933 and 1935, he and his associates administered to arthritic patients in the hospital and in private practice 6,000 ampoules of phenylcinchoninic acid in methenamine, and 63,000 capsules of cinchophen in methenamine and 33,000 capsules of neocinchophen in methenamine without a single instance of deleterious complication. Motzfeldt (243) noted in 1929 that 2,000 gms. of cinchophen were dispensed in 1927 in the Norwegian Hospital with only one case of jaundice, while none was observed in preceding years. Fleischmann (107) mentioned that in the Moabit Hospital in Berlin 8,000 gms. of cinchophen were prescribed in 1931 without a single therapeutic accident. Klemperer (196) was quoted by Fleischmann to the effect that 20 kg. of this drug were used within 2 years in the same hospital without any apparent ill effects. Snyder, Traeger, Zoll and Lust (335) administered cinchophen to 2,560 patients within 10 years and observed only one case of mild jaundice and a few cases of urticaria. Curschmann (70) prescribed cinchophen for over 20 years and observed only one case of mild liver injury. Schittenhelm (307) made similar favorable observations at the Medical Clinic at Munich.

Less favorable are the observations made at the Hertzler Clinic, according to the report of Westfall (389). Data were compiled from 1,589 cases, out of a total of 2,467 cases, treated with cinchophen (886 cases from 25 to 100 tablets, 195 cases from 250-500 tablets, 47 cases from 500 to 1000 tablets, 11 cases from 1000 to 1500 tablets). Several patients took the drug daily for 3 to 4 years.

One woman, who received 45 tablets, died with acute yellow atrophy, and two additional patients developed jaundice from which they recovered. It is significant also that Weir and Comfort (384) recently observed during the 10 years after 1923 nineteen cases of toxic cirrhosis due to cinchophen at the Mayo Clinic.

Additional evidence concerning the relation between the incidence of cinchophen hepatitis and the total amounts of this drug used was provided by White (391), who estimated that approximately 100,000 lbs. of cinchophen and its derivatives are consumed annually in the United States. Assuming that the average dose is 0.5 gm. he calculated that a total of 875,000,000 doses were given within 10 years. Since a total of 88 deaths from cinchophen have been reported during this period from U.S.A., Germany, England, and Austria-Hungary, there would be only one death for much more than 10,000,000 doses of cinchophen. Snyder and his coworkers (335), who attributed only 18 deaths to cinchophen and cinchophen preparations during the years 1913-1933, arrived at even a much lower figure, i.e., 1 death per 100,000,000 doses. The data supplied by Weir and Comfort (384), however, suggest that cinchophen may cause considerably more than one death in 10,000,000 doses. Von Oettingen (375) estimated in 1933 the occurrence of one therapeutic accident for 500,000 doses given.

It is obvious that the more optimistic of these estimates are understatements as to the degree of hazard present, as, doubtlessly, an unknown number of additional cinchophen accidents was not reported. In some of these cases the causal relationship probably was not recognized, while in others an incorrect diagnosis of the liver disease present may have been made, such as carcinoma of the liver (389). However, compensating at least in part for these considerations favoring a higher cinchophen toxicity is the fact that jaundice occurs in arthritic patients without the intervention of this drug (197). Sager (301) analyzing the incidence of jaundice in arthritic patients at the Mount Sinai Hospital in New York found that during the years 1921 to 1935 there were 10 cases of jaundice following the administration of cinchophen against 20 cases of jaundice which had not received this drug. Sager (301) concluded from these statistical data that cinchophen may have been accepted too readily as the causative factor for certain cases of jaundice, an opinion which was also previously voiced by Mallory (48). In view of the relative rarity of cinchophen hepatitis in gouty patients, it may be mentioned that jaundice is rarely seen in association with gout (103).

The incidence of cinchophen hepatitis is not large enough to be recognized in the statistical death rate of yellow atrophy of the liver, which has remained uniform for 35 years before and after the introduction of cinchophen and related drugs which have been introduced since 1902 (69, 46). The total clinical evidence thus does not support the concept that cinchophen exerts a direct chemotoxic effect upon the liver cells.

This conclusion is supported by the negative outcome of most of the experiments in which it was attempted to reproduce in animals by the repeated administration of toxic doses of cinchophen or neocinchophen hepatic changes such as those seen in man.

The following positive results were recorded. Biberfeld (29) reported in 1913 that a dog which had received 2 times 5 gms. of cinchophen died with hepatic lesions resembling those of acute yellow atrophy. Chen (58) stated in a brief discussion remark that dogs which had been given small to moderate doses of cinchophen showed at death, after several weeks on this management, an acute yellow atrophy of the liver. Surviving animals had at this time yellow colored conjunctival membranes and revealed later at autopsy similar hepatic lesions. The same changes were seen in dogs which had received for many months cinchophen in amounts which were about half of those given to the dogs of the first set. Churchill and Van Wagoner (62) observed in 3 dogs, which had been given for 8 to 10 days, when they refused to eat, 27 times the human therapeutic dose (595 mg./kg.) of cinchophen mixed with the food, first a marked rise in the urea N content of the blood and then a depression of this value. One of the dogs showed at autopsy an ulcer of the stomach and scattered coagulation necroses of the liver. Similar observations were made in a second series of dogs, in which the decrease in the urea N level was associated with a positive bromosulphthaleine test. When dogs were given 600 mg./kg. of sodium cinchophen by mouth, causing death after 3, 4, 9 and 21 days, Barbour and Fisk (9) found at autopsy yellow mottled kidneys and large yellow spotted livers of jelly-like consistency having extensive necroses of the hepatic parenchyma. Dogs which received daily doses of from 50 to 400 mg./kg. of cinchophen for from 4 to 33 days revealed necroses of the liver and tubular degeneration of the kidneys. Of several dogs which were given 600 mg./kg. of neocinchophen daily for 7 weeks, one showed at death widespread liver necroses, while of two dogs given 1 gm./kg. daily for 4 weeks one had diffuse necroses of the liver.

In rats which had been given 600 mg./kg. of cinchophen daily by mouth for 49 days, Barbour and Fisk (9) saw necroses of the liver. These were scattered in animals which were killed after 8 to 15 days on this treatment, but were diffuse in those which were sacrificed after 49 days. Rats which received 400 mg./kg. of the drug for 14 days were without liver pathology, while those which were given 800 mg./kg. daily revealed occasional necroses in the liver. Rats which were fed 1 gm./kg. of cinchophen by mouth exhibited moderate to diffuse necroses of the liver. Similar observations were made by Reichle (288) in rats which were subcutaneously injected with 1-2 gms./kg. of cinchophen dispersed in gum acacia solution. There were, on the other hand, no cirrhotic changes in the livers of rats kept on a diet containing 0.2 to 0.5 gm./kg. of cinchophen over prolonged periods. Similarly negative results were obtained when the livers were first injured by the treatment of the rats with chloroform followed by the administration of cinchophen. Knoble and Smith (200) injected subcutaneously rats with 250 mg./kg. of cinchophen for 12 to 32 days (total dose 641 mg. to 1,748 mg.) and found in seven of seventeen rats a mottled, yellowish brown liver containing marked cloudy swelling of the liver cells, hemorrhages, and, in a few instances, scattered necroses. Barbour and Fisk (9) concluded from the evidence available that the hepatotoxic action of cinchophen and its derivatives was definitely established.

However, when these investigators repeated their experiments on rats which were subjected to various dose levels of cinchophen and the hepatic resistance was in some of them weakened by the administration of chloroform or starvation, they did not succeed in obtaining in a single instance any degenerative changes of the liver. Even when 750-3,000 mg./kg. of cinchophen were given, the rats died without any evidence of liver damage. In view of these consistently negative results, Barbour and Gilman (10) were forced to admit that cinchophen hepatitis can not be experimentally produced at will. Lehman and Hanzlik (210) fed young rats a synthetic diet containing 0.5, 1 and 2.5 per cent of cinchophen and 1 and 2.5 per cent of neocinchophen. There was some retardation of growth, especially in the series receiving the diet with the higher concentrations of cinchophen. The livers of rats killed on the 17th day were normal. Radwin and Lederer (283), investigating the effect of high carbohydrate and low carbohydrate diets upon the toxicity of cinchophen in rats, found that rats placed on such diets and receiving up to 10 times the human therapeutic dose of cinchophen into the muscle exhibited, after various lengths of time on this treatment (up to 135 days), noncharacteristic liver changes, which did not resemble those of acute yellow atrophy. They concluded that cinchophen probably lowers the resistance of the liver to hepatotoxins.

In the report of Schering and Kahlbaum (303) mention is made of the fact that rats receiving 60 mg./kg. of cinchophen daily by mouth remained alive and healthy for many months. If 180 mg./kg. of cinchophen were administered subcutaneously daily for an additional three months to these rats no apparent harm was done. Rats which received 240 mg./kg. subcutaneously lived for 3 months, while those receiving 360 mg./kg. died within 14 days. The livers of these animals showed fatty infiltrative changes, but not necroses. Hueper (182) confirmed these negative findings on rats which received 60 mg./kg. daily by mouth with their regular food for a period of 3 months. When young rats were kept on a synthetic diet containing 2 per cent of gelatine which represented the only source of protein and which has a low methionine content (0.8 per cent), and received 0.06 gms. of cinchophen daily for 2 months, the livers exhibited only minor degenerative changes in general and were in a better condition than the livers of rats receiving the protein and methionine deficient diet only.

Klemperer (196) injected intravenously rabbits with 10 to 20 times the human therapeutic dose of cinchophen without producing any atrophy of the liver or other definite degenerative changes. He concluded that from this result one must be cautious in assuming that cinchophen generally produces acute yellow atrophy of the liver. Lehman and Hanzlik (210) gave cinchophen to rabbits by stomach tube, after having previously poisoned some of them with chloroform or phosphorus. The liver function tests of the last groups of rabbits improved during the subsequent period of cinchophen medication providing thereby proof that cinchophen did not aggravate the liver damage caused by typical liver poisons. Similarly negative results were observed in rabbits receiving ten times the human therapeutic dose (30 mg./kg.) of cinchophen for 45 days by Myers and Goodman (245). There were only minor liver lesions in these animals,

while the tests for urobilinogen and the van den Bergh tests were negative. Radwin and Lederer (283) following the example of Opie (256) who found that liver damage elicited by chloroform in dogs was accentuated by the simultaneous injection of bacterial cultures, gave coincident intramuscular administrations of a 10 per cent solution of sodium cinchophen and of cultures of living bacteria to rabbits without causing any liver injury.

Myers and Goodman (245) gave to dogs for 17 days 2 to 5 times the human therapeutic dose of cinchophen without eliciting appreciable liver injury. Ivy and coworkers (4) were unable to produce in dogs any lesions resembling yellow atrophy or cirrhosis when these animals received several times the human therapeutic dose of cinchophen by mouth. Only when very large amounts of cinchophen were administered were degenerative liver changes observed. These investigators concluded that cinchophen did not act like a hepatotoxin. Stalker, Bollman and Mann (341) never saw in their many dogs subjected to toxic doses of cinchophen any jaundice or liver injury. Hueper (182) administered to five dogs, weighing around 10 kg., a daily dose of 2.5 gms. of cinchophen. The animals died after 5 to 24 days, showing mild edema and degenerative changes of the liver. Similarly negative results as to the production of yellow atrophy, were obtained in 30 mice, weighing 25 gms., and given by stomach tube 10 mg., of cinchophen for a period of up to two weeks, and in two *M. rhesus* monkeys weighing 6.5 kg., and 8 kg., respectively, and receiving orally 1.5 gm. and 2.0 gm., respectively, of cinchophen by mouth for two to four weeks.

In judging the value of the experimental evidence, it is important to note that all experiments were performed with doses of cinchophen which are out of proportion to those used therapeutically in man. The effects obtained by this method in experimental animals are, therefore, not strictly comparable to the changes seen in man. The liver lesions described by some of the investigators differ distinctly in some instances from those characteristic of acute yellow atrophy; in others they may have been complicated by post mortem autolysis producing, thereby, a histological picture having perhaps a faint resemblance to that found in yellow atrophy. It is obvious that the bulk of the evidence obtained in mice, rats, rabbits, dogs and monkeys substantiates the conclusion of Lehman and Hanzlik (210), that there is no reliable proof that cinchophen is a hepatotoxic agent. The available experimental evidence indicates that cinchophen does not aggravate liver injury produced by previous chloroform or phosphorus poisoning. It does not interfere with the recovery from these conditions nor does it elicit such lesions if animals are kept on a diet low in carbohydrates or proteins, which are presumed to lower resistance to typical liver poisons.

With the elimination of a direct chemotoxic action of cinchophen upon the liver cells as the cause of the acute yellow atrophy and toxic cirrhosis observed in man after the administration of this drug, the possibility of a chemoallergic mechanism deserves serious consideration. Quick (278) suggested that a conjugation product of hydroxycinchophen with glucuronic acid might act as a hapten causing a sensitization of the organism, with the liver acting as the shock organ. Willcox (393) pointed out that idiosyncrasy plays an important role in

the production of toxic jaundice, such as that following the administration of arsenobenzol, gold salts, diethyl stilbestrol, plasmoquine, and quinoline derivatives. He suspected that a lack of nutrition of the liver cells is connected with the development of an allergic liver necrosis. Other quinoline derivatives than cinchophen (yatren) have occasionally elicited acute yellow atrophy (401, 175, 87). In favor of an allergic origin, mention may be made of the positive skin reactions occasionally obtained with cinchophen in persons with cutaneous and hepatic reactions. However, skin sensitivity does not necessarily accompany hepatic susceptibility. The wide variations in the dose of cinchophen previous to the appearance of toxic jaundice, and the erratic and scattered appearance of this reaction among patients treated with this drug, supply additional evidence supporting the allergic concept. It must be conceded, however, that the entire evidence is of purely circumstantial nature, and that the mechanism of the development of a chemoallergy to cinchophen leading to an acute yellow atrophy of the liver is as obscure as that of most other allergic drug reactions, which have become increasingly important in recent years, especially by the production of serious blood diseases.

There exists the possibility that a third mechanism may be active in the production of cinchophen hepatitis. In vivo experiments have shown that cinchophen medication causes a disturbance in the immune status of the body by acting upon the circulating and fixed antibodies. It is conceivable that such changes may help in the activation of latent hepatotoxic infections, such as those caused by viruses. The possible existence of such an interrelation is suggested by the fact that toxic jaundice has appeared in relatively few patients suffering from the metabolic disorder of gout, while it has affected mainly individuals with chronic infectious arthritis. Consideration also should be given to the possibility that such virus infections might have been occasionally transmitted by the use of improperly sterilized needles when cinchophen preparations or other medication were given parenterally, inasmuch as such accidents have occurred following the injection of insulin (86, 234). It may be mentioned in this connection that cinchophen does not exert any bactericidal properties against gram positive and gram negative microorganisms, according to the results obtained in in vitro experiments.

Despite the existing uncertainty concerning the actual mechanism which is responsible for the development of acute yellow atrophy and toxic cirrhosis of the liver in the cases reported, there can be little doubt that cinchophen therapy was in some way involved in the development of most of these complications. Every physician who prescribes cinchophen and every patient who takes this drug should be familiar, therefore, with this serious hazard, even though it affects only the exceptional individual.

E. CONTRAINDICATIONS TO THE USE OF CINCHOPHEN

In the absence of adequate evidence on which to base a reliable estimate of the relative dangers connected with cinchophen therapy, numerous contraindications to its use have been proposed. The following conditions have been

noted in this respect: previous and coexisting disease of the liver and gallbladder, especially jaundice, eclampsia, circulatory disturbances affecting the function of the liver and of the kidney, renal diseases which may interfere with the excretion of cinchophen and its metabolites, allergic disorders (hay fever; bronchial asthma; allergic dermatoses); malnutrition causing a depletion of the glycogen and protein contents of the liver (278, 73, 280, 386, 354, 219). Hocgler (175) mentioned that persons with hyperthyroidism were especially sensitive to cinchophen, while Schaffler (302) noted that the treatment of jaundice with cinchophen was irrational, as it had caused an aggravation of this condition in some cases (249). Quick (278) advised that during the treatment with cinchophen no foreign proteins or vaccines should be given, and Westfall (389) warned before the use of heavy metals (mercury) and arsenic in combination with cinchophen. Others mentioned iodides in this respect. The main reason for considering these diseases and therapeutic measures as contraindications to cinchophen therapy is the fear that they might increase the chances for the development of cinchophen hepatitis.

In general such conclusions are not based upon actual clinical evidence demonstrating the existence of these alleged interrelations, but they are drawn from an analogy with clinical and experimental observations showing that a diet low in carbohydrates and/or protein and high in fat content lowers the resistance of the liver to poisoning with typical hepatotoxins, such as phosphorus and chloroform, and that, on the other hand, diets high in carbohydrates and proteins and low in fat decrease the hepatic susceptibility to these poisons (257, 75, 239, 137, 177, 235). Himsworth and Glynn (172) demonstrated that rats kept on a diet deficient in protein and, particularly, in methionine, developed massive liver necroses, such as those seen in acute yellow atrophy in man.

It is doubtful, however, whether or not all these contraindications have any real value as far as the cinchophen hazard to the liver is concerned. All experimental observations on the aggravation of hepatotoxic dietary deficiencies and of toxic liver injury by subsequent cinchophen administration have been negative, as noted above. Mention, however, may be made of the fact that the gastrointestinal disturbances sometimes elicited by cinchophen and resulting in loss of appetite, vomiting and diarrhea, thereby producing a state of malnutrition, have been noted to precede the onset of jaundice in some cases. There is reason to believe also that other contraindications listed may have as much merit. It is, for instance, purely speculative that allergic disorders of non-cinchophenic type should favor the development of similar reactions by this drug.

As contraindications to the continued use of cinchophen, the following manifestations are listed: jaundice, itching, hives, rashes, nausea, vomiting, loss of appetite, vasoneurotic reactions, and similar untoward responses appearing after the administration of the drug and presumably caused by it. They are regarded as an indication for the immediate stoppage of any further cinchophen medication. Although there is some uncertainty in regard to the validity of some of these contraindications, it seems to be wise to adopt a conservative at-

titude in this respect as long as the present state of ignorance concerning the actual factors involved in the causation of cinchophen hepatitis exists.

F. PREVENTIVE AND THERAPEUTIC MEASURES IN CINCHOPHEN POISONING

Measures directed at the prevention of untoward and, especially, hepatotoxic reactions to cinchophen are of diagnostic, dietary, and medicinal nature. It is advisable to test, in intervals during and some time after the treatment with cinchophen, the urine for urobilinogen and the serum for its content of bilirubin, so as to discover the earliest signs of liver damage (283). Inasmuch as the impression prevails that a continuous medication with cinchophen favors the development of liver injury, most clinicians have adopted an intermittent type of treatment leaving free intervals between the courses so as to permit the liver to recover from any potential degenerative effects. It is recommended, moreover, to administer the drug in doses not exceeding 3 gms. per day and with large amounts of liquids so as to aid its excretion, to give sodium bicarbonate with it, so as to counteract any gastric irritation, and to feed a diet providing ample amounts of carbohydrates (sweetened lemonade, cake, flour, sugared fruits, etc.), so as to keep the glycogen content of the liver at a high level (44).

Quick (278) suggested that the detoxification of cinchophen be stimulated in the body by feeding glycine, as, in his opinion, this chemical is conjugated with cinchophen in this process. Hueper (182) introduced into mice by stomach tube lethal doses of cinchophen (25 mg. of the drug per 25 gm. mouse) and combined this treatment in additional series of mice with the administration of 25, 50, and 75 mg. of ascorbic acid, and of 25 mg. of ascorbic acid plus 25 mg. of cysteine hydrochloride, respectively, in an attempt to accentuate the detoxication of cinchophen. The experiment was repeated three times. There were no appreciable and consistent effects upon the mortality rate of the mice receiving the detoxicants compared with that in the control series. Similarly negative was the outcome of experiments in which sublethal toxic doses of cinchophen were given to mice, rats and dogs in conjunction with ascorbic acid and methionine over periods of several weeks. In no instance did the administration of the detoxicants exhibit any protective power against the toxic and ultimately lethal action of the drug. Peptic ulcers of the stomach were as frequent in the control dogs as in the dogs receiving 2.5 gms. of cinchophen plus 1, 2, and 3 times equivalent doses of ascorbic acid.

In evaluating the experimental data on the effect of detoxicants upon the lethal and toxic effects of cinchophen in various species of animals, it must be realized that the protective mechanisms of the human organism in many instances respond quite differently from those of the common laboratory animals, i.e., different species vary in their use of the available detoxicating agents (sulfuric acid, acetic acid, choline, glucuronic acid, glycine, glutamic acid, cysteine, methionine, ornithine, etc.) for the metabolism of the same chemical poisons (313).

Rawls (285, 286) reported that patients receiving vitamin K and bile in conjunction with cinchophen revealed a distinctly lower incidence of toxic reactions

of the skin, gastrointestinal tract, and nervous system than patients treated with cinchophen only.

When toxic jaundice once has developed it has become the rule during recent years to administer dextrose solution by vein and proper amounts of insulin subcutaneously, so as to stimulate the defensive power of the liver (72, 51, 93). So far no practical use, apparently, has been made of methionine medication in cases of cinchophen hepatitis, since the injection of solutions of this amino acid allegedly has been beneficial in several cases of liver injury caused by chlorinated hydrocarbons.

It is apparent from these considerations and observations that patients receiving cinchophen therapy deserve close medical supervision so as to reduce to a minimum the possibility of fatal therapeutic accidents. If the proper precautions are taken, the medicinal use of cinchophen or neocinchophen appears to be as safe as that of many other modern and valuable chemotherapeutic agents which have given rise to occasional dangerous drug reactions. Cinchophen seems to compare favorably, for instance, with gold compounds which were introduced for the treatment of arthritis. Snyder (334) stated that the gold therapy of arthritis causes, not infrequently, untoward reactions which are serious in 3 per cent of the cases, according to his own observations, and in 6 per cent, according to other investigators. Snyder (334) estimated that 1 per cent of these cases probably end in death, while Bauer noted a therapeutic fatality rate of one death in every 200 cases treated.

G. THERAPEUTIC USE OF CINCHOPHEN

Cinchophen and its derivatives have been employed for a wide variety of disease conditions. However, for certain purposes [choleretic (44, 45, 179, 148); contrast medium for gall bladder visualization (276, 315)] they have been replaced by more efficient and less dangerous medicinal agents. Cinchophen and cinchophenates are still used for their uricosuric, antipyretic, analgesic, and antiphlogistic properties.

Gout: Cinchophen is the first new remedy for gout since the introduction of colchicin preparations during the Middle Ages. Westfall (389) considers it the remedy *par excellence* for this condition. It has been used and it is still used for this disease on an extensive scale and with recognized success as a preventive of acute attacks as well as an abortive during an attack, where it brings symptomatic relief from pain and from swelling and redness of the affected joints within 24 hours (367, 382, 164, 165, 138, 51, 176, 118, 15, 307, 20, 196, 16, 290, 293, 189, 163). However, it apparently has no effect, or very little, upon the mobilization of the urates in the tophi (51, 332). It has been stated that cinchophen should not be administered during an attack of gout as the elimination of uric acid takes place as rapidly as possible and cannot further be stimulated by the drug. This conception of the antigout mechanism of cinchophen rests upon the belief that the beneficial action of cinchophen is mainly an uricolytic one. It is not likely that this interpretation is entirely correct inasmuch as Klemperer (196), and Lublin (220) emphasized the fact that they observed

favorable results upon acute attacks of gout from derivatives of cinchophen which did not possess any uricolytic qualities. The antiphlogistic action seems to play an important role. The rationale of the mentioned recommendation is therefore doubtful.

Hench (164) administers during an acute attack of gout 0.5 gm. of cinchophen 3 to 4 times daily for 3 days or 0.3 to 0.5 gms. of neocinchophen 3 times daily in conjunction with a consumption of at least 2 liters of water, a medication of alkalies, and a diet rich in carbohydrates. Weintraud (382) gives a total of up to 10 gms. of cinchophen within 3 to 4 days and discontinues, then, its medication as the effect of cinchophen wears off. Schittenhelm (307) recommends the administration of 0.5 gms. of cinchophen 3 to 4 times daily for 3 to 4 days, followed by a cinchophen-free interval of 8 days.

Graham (138) observes the following scheme of cinchophen therapy for the prevention of acute attacks and for the relief of the chronic joint conditions: 1.25 gms. of cinchophen 3 times daily for 3 days, followed by a rest period of 3-4 days; then start of another course. Several of his patients have followed this management for several years and have consumed up to 600 gms. of the drug without bad effects. Weintraud (382) emphasizes that the use of cinchophen is especially indicated during the early part of the disease so as to prevent the development of the disabling articular and renal complications. Sherwood and Zimmerman (323) recommend the use of wine of colchicum during the acute attack until intestinal symptoms appear. After relief from the acute symptoms is obtained, they give 1 gm. of cinchophen 4 times daily for one week, with 0.5 gms. daily during the second and third week; then they omit one day from the daily routine of cinchophen medication during the following week and increase the omission by one day each successive week.

Hollander (176) proposes, for the prophylactic treatment of gout, the administration of 0.5 gms. of neocinchophen 3 times daily for 2 to 3 days each fortnight. He believes that a more continued medication of cinchophen is inadvisable since the uricosuric effect of the drug weakens after 3 days. Baronigian (15) develops the following schedule of intermittent treatment of gout: for 6 days daily 0.5 gms. of cinchophen after meals 4 times daily together with 4 times 1 liter of water; following a rest period of 15 days, 0.5 gms. of the drug are given 3 times daily for 8 days together with 3 liters of water; after 2 weeks of rest, 0.5 gms. of cinchophen 2 times daily for 12 days together with 2 liters of water; following a rest of 30 days, 0.5 gms. once daily for 24 days with water ad libitum. Annegers, Snapp, Ivy and Atkinson (5) place the daily dose of cinchophen in gout at 7-28 mg. per kg.

It may be mentioned that Daniels (71) recommended that cinchophen be combined with lithium salts in the treatment of gout so as to accentuate the mobilization of urates in the tophi. Argentin and Ratti (7) combined cinchophen (4 gms.) with 800 units of ascorbic acid and claimed an improved effect upon the manifestations of gout.

Rheumatic Polyarthrititis: This disease represents the second important therapeutic indication of cinchophen and its derivatives. Hanzlik (154) noted that

cinchophen and congeners act upon rheumatic infection like the salicylates, and effect a rapid disappearance of redness and swelling of the joints, a marked reduction in body temperature and a production of abundant perspiration in many, but not all, instances. Some clinical observers considered neocinchophen, cinchophen or atophanyl superior, in their antirheumatic action, to aspirin 2, 24, 251, 196, 188, 152, 255, 57. Several investigators noted that cinchophen helped in cases in which salicylates failed to bring relief (24, 359). Hanzlik, Scott, Weidenthal and Fetterman (155) did not find cinchophen superior to salicylates in the treatment of acute rheumatic infection, while Pfeffer even found it inferior to the salicylates.

Miller and Boots (238) noted that neocinchophen was less effective in its antipyretic action than cinchophen. Swift (358) and Neukirch (251) stated that relapses in rheumatic fever occurred more often after cinchophen treatment than after salicylates, while McLester (227) reported failures of cinchophen in the treatment of rheumatism. Fischer (106) reported prompt and good results especially in regard to the relief of the arthritic symptoms from the use of atophanyl. Good effects were recorded by Brahn (39) who used leukotropin in 35 cases of rheumatism and noted the regression of pain within 3 to 10 minutes. Bendix (24) found methyl- and methoxy derivatives equally effective in 100 cases, 45 of which were completely cured. Klemperer (196) asserted that the antiphlogistic action of cinchophen is responsible for the antirheumatic effect. There is no evidence that cinchophen prevents the development of the rheumatic lesions of the heart valves (154).

Barbour, Lozinsky and Clements (12) recommended the following regime: 2 gms. of cinchophen every hour for 3 hours, followed by 1 gm. every 4 hours (4-5 times a day). A total of 10-16 gms. are usually needed to bring about complete relief. Some cases, however, require up to 50 gms. of cinchophen. Hanzlik, Scott, Weidenthal and Fetterman (155) obtained partial relief after 3-6 gms. of cinchophen, and complete relief after 10-13 gms. of cinchophen, with depression of temperature occurring at the end of 8-12 hours, or 16 gms. of neocinchophen, with depression of temperature appearing at the end of 24 hours. Boots and Miller (35) administered in some cases total doses of neocinchophen up to 282 gms. within 32 days, and 440 gms. within 118 days. Cecil (54), on the other hand, recommended the use of cinchophen only when other remedies have failed.

Erythema Nodosum: Ehrlich (91) reported prompt relief in one case of erythema nodosum within 24 hours after the intravenous injection of leukotropin.

Arthritis: Chronic arthritis has offered in the past years a wide field for the therapeutic use of cinchophen and is still employed for this purpose, especially when other therapeutic agents failed to bring relief from pain. It exerts here its beneficial effect by its analgesic and antiphlogistic action, but has no influence upon the basic causal mechanism of the joint condition (349, 88, 390, 371, 275). Eaton (88) prescribes, in chronic arthritis, 0.5 gms. of cinchophen or neocinchophen plus 0.5 gm. of methenamine in capsules, 1-2 capsules 3 times daily for 3

days, or 0.5 gm. of phenylcinchoninic acid in 10 cc. of a 34 per cent methenamine solution, 1 to 2 times weekly. Stones (349) gives 1 gm. of cinchophen 4 times daily, sometimes continuously, over long periods. Even obstinate cases of chronic arthritis may show temporary relief by this treatment (390).

Miscellaneous Diseases: Smith and Hawk (332) saw good results from cinchophen medication in inflammatory conditions of the eye. Mindana and Del Grande (240), and Graf (136) found that cinchophen does not definitely influence dermatoses associated with an increased uric acid level of the blood. Cinchophen and its derivatives have been used extensively as analgesics in the treatment of all kinds of painful conditions, such as neuritis, lumbago, ischias, muscle pain, pleurisy, toothache, etc. with varying success.

H. MEDICINAL STATUS OF CINCHOPHEN

The occurrence of serious therapeutic accidents in the form of toxic jaundice with not infrequent fatal outcome and the publicity connected with these complications have discouraged many physicians from using this drug. However, the professional opinions concerning the relative merits of cinchophen and its congeners as medicinal agents are sharply divided, and depend mainly upon the individual estimation of the degree of hazard connected with their therapeutic use.

A completely negativistic attitude is assumed in this respect by Comroe (66) who states that the high mortality attending cinchophen hepatitis and the lack of therapy argues against the use of drugs containing cinchophen or its derivatives. A similar judgment is passed by Myers and Goodman (245) who hold that therapeutic employment of cinchophen is not justifiable in view of the evidence of its toxicity. Fantus (101) asserts that the use of cinchophen should be abandoned and neocinchophen should not be employed in patients offering the above mentioned contraindications. Carroll and Elliott (51) conclude that, in view of the dangers incident to the administration of cinchophen, the unknown susceptibility of individuals to its toxic effects and the length of time which may pass after its administration before toxic manifestations appear, it seems unwise to administer cinchophen as a therapeutic agent under any circumstances, especially since many harmless and equally effective analgesic drugs are readily available for use. Bauer and Klemperer (20) want to exclude cinchophen from any further therapeutic employment in the treatment of gout.

An intermediary position permitting a restricted use of cinchophen containing drugs is taken by other investigators. In an editorial remark to a paper of Hench, Bauer, Boland, Dawson, Freyberg, Holbrook, Key, Lockie, and McEwen (165) it is stated that cinchophen never should be given for any type of arthritis except gout, and then only when gout cannot be controlled adequately by other measures. Some clinicians would not even use cinchophen under such conditions. A similar position toward the therapeutic employment of this drug is taken in an answer to a query which appeared in the *J. A. M. A.* (97:409, 1931), in which the following statement is made: "In view of the serious though rare poisoning from ordinary doses of cinchophen, the use of this drug should be

restricted to cases in which other non-narcotic analgetics, such as salicylates, acetylsalicylic acid or amidopyrine, have been tried and failed to give adequate relief, and in which the suffering is sufficiently great to justify the risk. Even then, it would be advisable to use neocinchophen". Lehman and Hanzlik (210) note that the legitimate and ordinary therapeutic medication, including the intensive treatment for rheumatic fever, given under the direction of a physician, is generally safe and capable of producing well-known benefits. They believe that the undesirable consequences of far-fetched strictures of a useful remedy are the dangers of medicolegal complications resulting from legitimate medication (381). Graham (138) is very loath to suggest that cinchophen should be abandoned for the treatment of gout as the results are so good in the great majority of gout patients. Palmer (260) advises that its use be restricted to gout patients, who are able, with rare exceptions, to take cinchophen for years without difficulty. Westfall (389) concludes from an analysis of the effects of cinchophen on a series of 2,467 patients with arthritis, lumbago, neuritis, and gout that the risk is small compared to the benefits obtained from cinchophen therapy.

Waggoner (371) comments that it is logical to believe that there may be an idiosyncrasy to cinchophen, just as there is to many other valuable drugs, and that it should not be discarded because of the unfortunate reaction of a few cases. He asserts that these drugs may be prescribed safely when a proper indication for their use exists. Comfort (65) voices the opinion that in the face of many deaths the continued use of cinchophen must be justified on definite grounds that in a particular case its use alone gives relief or much greater relief than less toxic analgetics. Hench (164) concedes that the administration of cinchophen to patients with gout carries a definite, while small, risk which some physicians never condone, but which most students of gout consider justifiable if gout is uncontrollable otherwise. He argues that the risk taken must be contrasted with that of the disease itself: in from 10-20 or more per cent of cases gouty nephritis develops which sometimes is fatal, and the incidence of renal colics from urate gravel and of cardiovascular lesions is notable. Since the disease thus carries a much greater risk than its treatment with cinchophen, it seems justifiable, in the opinion of Hench, to assume the small risk of the latter, when necessary, to try if possible to prevent the articular, renal and vascular consequences of gout. He insists, however, that patients should be told of the risk of toxic reactions from cinchophen and should be instructed to report to their physicians any of the various toxic manifestations listed above as contraindications for the continued use of this drug, so that counter-measures can be instituted at an early moment.

Schittenhelm (307) believes that the hazard of fatal injury from cinchophen is so minimal that any restrictions to its therapeutic use are not justifiable. He does not favor having cinchophen placed among the drugs obtainable only by prescription, as advocated by Cohen and others.

In 1941 Klumpp (199) attempted to obtain a cross section of medical opinion on the use of cinchophen by addressing a questionnaire with 4 questions on this subject to the full professors of medicine, pharmacology and pathology and the

clinical professors of medicine of the class A schools, as well as to those who had contributed to the literature on drugs. To the questions "Do you consider cinchophen and cinchophen derivatives indispensable therapeutic agents in the physician's armamentarium?" 217 or 82 per cent answered "No", 36 or 14 per cent answered "Yes", while 13 answers or 4 per cent were disqualified. To the second question "In your judgment do cinchophen and cinchophen derivatives have any essential therapeutic effect which cannot be accomplished more safely by properly regulated doses of other medicaments?", the answer was "No" in 189 cases or 71 per cent, "Yes" in 63 cases or 24 per cent, while 14 answers or 5 per cent were disqualified. The third question "Can cinchophen and cinchophen derivatives be administered in therapeutically active doses by physicians or by others with confidence that serious deleterious effects will not supervene?" was answered by 211 or 79 per cent with "No", by 34 or 13 per cent with "Yes", while 21 answers or 8 per cent were disqualified. The answers to the fourth question "Can the pathology of cinchophen poisoning be counteracted or cured by specific measures once the symptoms of poisoning have appeared?" were "No" in 205 cases or in 77 per cent, "Yes" in 28 cases or 11 per cent, while 33 cases or 12 per cent were disqualified.

The relative value of the opinions expressed in the answers to this questionnaire rests on the actual, well considered and controlled experience which the individuals expressing these opinions had with these chemotherapeutic agents. From an analysis of the data published in the medical literature, one is inclined to agree with a statement made in an editorial published in 1941 in the J. A. M. A. in which it is noted that there is an urgent need of controlled clinical studies of those conditions in which cinchophen is used, of its relative dangers, when contraindications are observed, and of the treatment of poisoning induced by cinchophen or allied drugs.

The evidence presented makes it advisable that the use of cinchophen and its derivatives for the treatment of ordinary colds, headache, neuritis, and similar conditions, requiring analgetic agents, should be entirely discontinued, since the risk entailed by the disease does not justify the administration of a medicinal agent carrying with it a serious potential hazard. On the other hand, the further employment of cinchophen and its congeners, with the exception of the iodine containing compounds, should be retained for the management of gout, rheumatic infection, and chronic arthritis, whenever these diseases cannot be satisfactorily controlled with less dangerous drugs. When cinchophen and its derivatives are given with proper precautions, the physician carries no more liability in the prescribing of these drugs than he does of any other potent agent.

It may be mentioned, finally, that laws were enacted in several States (California, Connecticut, Florida, Massachusetts, Nevada, North Carolina, and Vermont) prohibiting the retail distribution of cinchophen except on the written prescription of a licensed physician, dentist, or veterinarian (J. A. M. A. 1940). It is likely that similar regulations have been put in force in other States and countries by this time.

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POTASSIUM AND PERIODIC PARALYSIS

A METABOLIC STUDY AND PHYSIOLOGICAL CONSIDERATIONS

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It is paradoxical that knowledge of the pathological physiology in familial periodic paralysis remains incomplete despite the fact that successful treatment for this affection has been available since the empirical discovery of the value of potassium salts by Singer and Goodbody (82) in 1901, Buzzard (14) in 1901 and Mitchell, Flexner and Edsall (72) in 1902. The rarity of the disorder usually limits metabolic research efforts to the study of single patients. Such a study is reported here with special attention to disturbances in potassium metabolism.

This paper presents a brief summary of present concepts of the metabolic defect in periodic paralysis, a recording of our experimental procedure, results and their significance, and a short review of potassium metabolism.

In 1934, Biemond and Daniels (6) noted incidentally that the serum potassium level during an attack of paralysis was low. Since the first report dealing specifically with the role of potassium in familial periodic paralysis by Aitken, Allott, Castleden and Walker (1) in 1937, there have been several such metabolic reports in the literature (2, 39, 43, 77, 78, 89). Except to establish the important role that potassium plays pathogenetically and therapeutically in this disease, little more information about the faulty metabolism in familial periodic paralysis, except in a negative way, has been disclosed.

The clinical picture of this disease has been clearly described many times, especially by Talbott (89) in whose paper over 400 cases in the literature are reviewed. The clinical picture in our patient is typical with the exception of the absence of a history of a familial incidence. Sporadic cases of periodic paralysis are not unknown. Herrington (50) reports an incidence of 20 per cent of non-familial cases. There is ample clinical and chemical evidence in this and other cases to challenge the contention of Holmes (53) that such isolated instances are hysterical.

It is of incidental interest that World War II has in one way or another brought to light several recent cases (53, 55, 94, 95), presumably because the greatest reservoir of the disease, young male adults, received more medical supervision during war years. Although the disease in the subject of this report was not recognized in the service, it manifested itself there like others, for the first time. This may be explained by the fact that these susceptible patients were subjected to extraordinary stresses during military service. These sometimes seemed to precipitate attacks. In this case, prolonged ditch-digging immediately preceded the first attack.

In a recently reported case (95), that of a soldier who was thought to be psychoneurotic, an unusual feature, which has been noted occasionally before, was the presence of normal serum potassium levels during an attack of paralysis. This is not in agreement with most previous reports (1, 39, 43, 51, 77, 89). According to these, the development of an attack of paralysis coincides with a falling serum potassium level, and recovery, with a rising potassium level. This decrease of potassium was never accompanied by increased potassium excretion. As a matter of fact, it has been shown that just before and during an attack the potassium excretion may be lower than normal. Where the potassium migrates during an attack is an unsolved problem.

Except with regards to phosphorus, no other significant electrolytic change has been noted during an attack of paralysis. Although Ferree, Gerity, Atchley and Loeb (39) and others have failed to confirm it, Allott and McArdle (2), Talbott (89) and Hildebrand and Kepler (51) found that during the development of an attack and recovery from it the serum inorganic phosphate changes are similar to those of serum potassium but are less marked. Here, too, there was no increased excretion of the serum phosphates during paralysis. Milhorat (67), however, found a negative phosphorus balance following attacks.

Previous reports have not stressed sufficiently the clinical and chemical changes in the initial stages of an attack. Such studies necessitate experimental induction of attacks. Many methods of artificially producing them have proven successful. The most reliable of these has been that of feeding the patient a large amount of glucose. This, it has been suggested, simulates the natural circumstance of a large evening meal followed by the development of an attack of paralysis during the night.

Our experimental procedure consisted of close chemical and clinical observations of our patient during a preliminary normal period and during two successive artificially induced attacks. The second of these was produced in relation to a tracer experiment with radioactive potassium (K^{42}). An attempt was made to determine by body surface and body fluid readings with a Geiger-Mueller counter after the injection of K^{42} whether an abnormal shift in marked potassium could be detected in liver, muscle, serum, red blood cells, urine or spinal fluid of the patient as compared to a control, following the induction of an attack. The results in this portion of the experiment, however, were largely inconclusive due to certain physiological and technical limitations (short penetrating power of beta radiation given off by K^{42} , the limiting safe dose of K^{42} and the masking effect of the high background content of potassium in muscles). Consequently, except for a few observations of possible significance or of incidental interest recorded below, a detailed account of the tracer experiment has been omitted.

CASE HISTORY

FIRST ADMISSION

History: This 23 year old white male, N. K., M.H. #40782, was first admitted to Montefiore Hospital on July 18, 1946 complaining of lack of power in all four extremities and back.

He had always been in good health until three years prior to admission when, while undergoing basic training in the Army, he had the first of four similar subsequent attacks characterized each time by profound weakness of both arms, both legs and back, noted first when attempting to rise out of bed. He would awake feeling well, but to his surprise, when he attempted to move, he found that his limbs were almost powerless. He required assistance to get out of bed or to rise from a sitting to a standing position. Once erect he could maintain himself, although in a somewhat slumped posture. A shuffling gait with this slumped posture was possible, but raising his legs as in climbing stairs or for other reasons could not be done. His upper extremities were similarly affected so that they could not be raised even to his shoulder level. His grips would be markedly weakened as well. This state would persist for 24 to 48 hours, and then it would gradually, spontaneously clear. The whole attack from beginning to end would last 2 or 3 days.

There was never any disability, however, in speech nor mastication, nor in holding his head erect, nor in respiration. Bladder and bowel function remained intact and libido and potency were unaffected during attacks. Mental faculties were unimpaired, and there were no episodes of unconsciousness. Paresthesiae, diplopia, facial weakness, ptosis or dysphagia did not occur. Occasionally, a day or two after an attack he noted soreness in his calf muscles.

The first two attacks followed activity somewhat unusual for him. The first developed on the day following ditch-digging and the second, a year and a half later, after a 17 hour train ride. The third attack occurred one week before admission. On the day before being admitted, he developed the fourth attack. This one began a little more insidiously than the others manifesting itself first by some weakness in attempting to climb stairs in the afternoon; this was followed a few hours later by weakness in his left leg when attempting to step on the clutch pedal. The next morning he was in a fully developed attack.

The family history did not reveal any illness of neurological or metabolic interest. No other members in his immediate or distant family suffered similarly as far as could be determined.

Except for a history of ragweed hay fever since the age of ten and bronchopneumonia at thirteen his own past history was of no particular interest.

Clinical Examination: The blood pressure was 120/80; temperature, 98.6; pulse, 88 and regular; respirations, 20.

Upon admission the patient was in the midst of an attack. His physical habitus was muscular and stocky. He seemed to be in robust health. He could not maintain a sitting position unsupported, and when helped into a standing position, he maintained it in a lordotic posture with rounded slumped shoulders. Short, shuffling steps were possible. The calf musculature was very well developed. There was marked weakness of the trunk and limb musculature. The muscular weakness was most marked proximally and on the left side. He could not raise his arms to his shoulder level; nor could he hold his fingers in extension. Although it was possible to raise the right leg from the bed with great effort, he could not lift the left one at all. Strength in the feet was only slightly impaired. The left hip flex reflex and the right triceps could not be elicited. The suprapatellar and the patellar reflexes were depressed on the left. The abdominals and the right cremasteric reflexes could not be elicited. The plantar responses were promptly flexor.

Laboratory Studies: The blood sugar was 86 milligrams per 100 cubic centimeters; blood urea nitrogen, 13.7 milligrams per 100 cubic centimeters. The hemoglobin was 105 per cent (15.3 grams); red blood cell count, 5,210,000 per cubic millimeter; white blood cell count, 5,210,000 per cubic millimeter; white blood cell count, 7,950 per cubic millimeter with a differential count as follows: stab 3, segmented 63, lymphocytes 29, monocytes 5. Urinalysis showed a specific gravity of 1.017, an acid reaction, no albumin or glucose, and a sediment which was clear except for calcium oxalate crystals and mucus threads.

The lumbar puncture, which was done in the lateral recumbent position, was normal except for an initial pressure of 170 millimeters of water which fell to 150 millimeters after removal of 10 cubic centimeters of clear colorless fluid. There was no evidence of mano-

metric block. The spinal fluid protein was 60 milligrams per 100 cubic centimeters. No cells were found. The spinal fluid and blood Wassermann tests were negative; the gum mastic curve showed no significant alteration (0011100).

The serum potassium on the day after admission, the second day of a severe attack, was 3.3 milliequivalents per liter (12.8 milligrams per 100 cubic centimeters). The 24 hour output of creatinine on July 25, 1947, when the patient was nearly well, was 1.21 grams, while the creatine output was 0.09 grams.

Roentgen rays of the spine were normal except for bilateral sacralization of the fifth lumbar vertebra.

Course: On July 19, 1946, he was started on 8 grams of KCl daily. One half hour after the third dose, after a total of 6 grams of KCl, he began to feel improved. On July 20, he was practically fully recovered. He remained well until July 24 when KCl was stopped. Within a few hours he began to feel another attack coming on. Serum potassium at this time was 3.7 milliequivalents per liter (14.4 milligrams per 100 cubic centimeters). He was discharged on July 29, 1946 on KCl medication and advised against excessive carbohydrate intake.

SECOND ADMISSION

He was readmitted on September 4, 1946 to the Montefiore Hospital. He stated that since his discharge from the hospital in July he had been taking 5 grains of KCl three times a day until ten days prior to readmission when he stopped. Up until that time he had felt well and free of any symptoms. Two days after stopping the KCl he had a minor attack limited to slight weakness of his left lower limb. During the next four days he took varying amounts of KCl medication. After two days this focal weakness disappeared.

Clinical Examination: The patient was suffering from ragweed hayfever upon this admission. The blood pressure was as follows:

	Right arm	Left arm
Standing.....	170/124	160/120
Sitting.....	138/104	154/114
Supine.....	150/104	150/110

The blood pressures recorded represent the lowest of several consecutive readings.

His gait was normal except for evident weakness of the left lower limb when walking on his toes. Further tests of muscle strength revealed slight weakness in the fingers of his left hand, in both hand grips, in the flexors of left hip and knee, and of left plantar flexion. (The patient was left handed.)

The left patellar and ankle jerks were less active than the right. The abdominal and cremasteric reflexes were not elicited on the right side.

EXPERIMENTAL PROCEDURE

The patient was maintained on a diet which was controlled for potassium, phosphorus, caloric and carbohydrate intake. From September 4 to September 9 the potassium intake was kept between 3.4 to 4.2 grams daily and was varied somewhat between these limits in an attempt to keep him clinically free of symptoms. The phosphorus intake during this time varied between 0.3 and 1.3 grams. The caloric intake in this interval was kept below 2500 calories, and the carbohydrate daily intake was kept under 200 grams. For the first two days only, through September 6, the dietary potassium and phosphorus contents were estimated from tables by Sherman.* On the evening of September 6, he was started on a diet consisting of milk, cottage cheese, bread and a mixture of amigen and glucose supplemented by nicotinic acid, thiamin and riboflavin so that the intake of phosphorus and potassium could be more easily controlled and more accurately measured. Aliquote portions of the milk and of the amigen-glucose mixture were taken and samples of the bread and cottage cheese were ashed. These were all analyzed for potassium and phosphorus content

and the daily intakes of these elements were accordingly calculated. In order to minimize errors the bread and the cottage cheese, the only solid items of food, were limited so that the potassium intake from this portion of his diet was less than 0.5 grams.

* Sberman, H. C.: Chemistry of Food and Nutrition, New York, MacMillan Co., 1941.

Fractional urines, which were analyzed for potassium, inorganic phosphorus, creatine and creatinine content, were collected daily as follows: from 7 a.m. to 7 p.m., from 7 p.m. to 11 p.m., and from 11 p.m. to 7 a.m. These hours were chosen because clinically, both in his case and in the typical case described in the literature, attacks develop during the night and are evident on rising in the morning. Consequently, the most important metabolic changes might be expected to occur between the supper hour of one night and the early hours of the next morning. For the same reason routine blood specimens, for serum potassium, sodium and inorganic phosphorus and for whole blood potassium, were drawn at 10 p.m., 2 a.m. and 7 a.m. The red blood cell concentration of potassium was calculated as the difference between the whole blood potassium and the serum potassium. Allowances were made for hematocrit changes where necessary. Stools were analyzed for potassium and phosphorus. The experimental day was designated as extending from 7 a.m. of one morning to 7 a.m. of the next morning. Sodium and potassium determinations were made by flame photometry through the courtesy of American Cyanamid Company and Dr. John W. Berry of that organization by the use of their improved photometer which operates with an accuracy of about 1½% for sodium determinations and about 1% for potassium determinations (4), and which has been found satisfactory for biological analyses (46).

Evaluation of the patient's clinical status was made frequently. A system was devised for grading the depth of an attack by estimating the amount of impairment of each of several functions, such as walking on toes, chair stepping, muscle testing, dynamometer readings matched against a constant control, reflexes, general subjective and general objective status, respectively. Each of these functions were graded 0 to 4; 0 being complete loss of function and 4 being no loss of function. The sum of the values obtained at a given evaluation was calculated against the sum which would have existed if each function were graded as 4. (Not all functions were evaluated at every testing, but usually most of those listed above and often all of them were tested.) By such a comparison with a theoretical normal response for the patient, the percentage of attack existing at the time of evaluation was calculated.

Certain other isolated physiological and clinical determinations were made, including spinal puncture, both before and after an attack had developed.

On September 9 at 10:30 p.m., after he had been relatively free of symptoms for at least 4 days of observation, and after repeated routine observations of the chemical constituents of blood and urine during this time (Table I) and just following the taking of a preliminary blood specimen, an attempt was made to induce an attack of paralysis by feeding orally 300 grams of glucose dissolved in one pint of water. Blood specimens were drawn at 15 minutes, ½ hour, 1 hour, 2 hours, 3 hours and 8½ hours after this. These were analyzed routinely and in addition for glucose levels. The clinical condition was followed carefully thereafter. A complete attack resulted. Routine urines continued to be collected. Blood specimens were taken when the attack was fully developed.

At 3:30 p.m. of the following day (September 10) an attempt was made to reproduce the Pudenz experiment of injecting 1 gram of 1% KCl solution intravenously in one arm while maintaining the opposite arm occluded arterially (77), but the patient could tolerate only ½ gram in his vein and this had to be discontinued. No improvement resulted from this amount. It was then attempted to bring him out of the attack by feeding KCl orally. Within a few hours of this he showed marked improvement clinically. By the afternoon of September 11, he was fairly well out of his attack.

The second attack was induced during the night of September 11. A control subject of about the same age and weight as the patient had been prepared for the preceding 2 days

TABLE I

Summary of experimental data

DAY	TIME	CLINICAL CONDITION Per cent of maximal attack	K INTAKE			K OUTPUT			P INTAKE			P OUTPUT			WHOLE BLOOD K mgms. per cent	SERUM K mEq/l	SERUM P mEq/l	SERUM Na mEq/l	BLOOD GLUCOSE mgms. per cent	SPINAL FLUID		URINE CREATINE gms. per 24 hrs.	URINE CREATININE gms. per 24 hrs.	URINE OUTPUT c.c. per 24 hrs.	BLOOD COUNTS					BLOOD PRESSURE	EKG, PULSE																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
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9/7/46 # 4	7 am 7 30 am 1 30 pm 10 pm 2 am	12 5 4 0			175 5 3 7 139	54 50 55 4 5 20 7 6 5	90 regular
9/7/46 # 5	7 am to 7 pm 7 pm to 11 pm 11 pm to 7 am 24 hr total	8 0	4 30 2 58 03 2 89 41 49 02 51 71 12 12 40 40 10 19 4 30 3 97 03 4 00 41 50 02 82	41 49 02 51 71 12 12 40 40 10 19 41 50 02 82	175 5 3 9 1 9 171 5 3 7 1 6 133 171 5 3 0 1 7 136	106-170/118-122 (tense)	
9/8/46 # 6	7 am 2 pm 8 15 pm 10 pm 2 am	20 12 5 19	3 78 2 83 28 3 78 3 87	2 83 29 54 76 15 28 09 3 87 20 78	171 5 3 6 1 8 130 173 5 3 7 1 9 61	144 148/102-104	
9/9/46 # 7	7 am to 7 pm 7 pm to 11 pm 11 pm to 7 am 24 hr total	20 12 5 19	2 78 1 33 22 2 78 1 55	1 33 27 35 22 14 1 55 27 49	171 5 3 5 1 9 139 168 0 2 9 1 6 131 168 0 2 0 1 2 133 111		
500 grams glucose orally							
9/9/46 # 8	10 45 pm 11 pm 11 30 pm 12 30 am 1 15 am				171 5 3 5 1 9 139 168 0 2 9 1 6 131 168 0 2 0 1 2 133 111		90 regular
9/9/46 # 9	1 30 am 1 35 am 1 50 am 2 30 am				169 5 2 6 1 4 133 132		bigem pulse 84 regular bigem pulse
9/9/46 # 10	10 30 pm to 7 am		31	31		140-150/ 90- 04	

TABLE I—Continued

[illegible]

9/11/40 (Cont'd)	500 grams glucose orally										180/60	bigem EKG
8 02 to 5 03 a m												bigem pulse 104 regular
0 10 a m												
5 20 a m												
5 33 a m												
6 05 a m												
6 25 a m												
7 03 a m												
7 40 a m												
8 20 a m												
7 p m to 8 30 a m												
25 1 hr total												
0/12/40	9 a m											
# 0 0a m to 4 30 p m	11 10 a m											
	4 30 p m											
0/14/40											104/64	
0/18/40											80/40-50	50 regular

with an identical diet. From about 9:15 p.m. until glucose induction about 7½ hours later both patient and control were subjected to almost continuous body readings with the Geiger-Mueller counter, prolonged lumbar punctures and repeated venipunctures for chemical and radioactive determinations associated with the K^{42} experiment. At 9:30 p.m. 180 milligrams of potassium which included about 144 microcuries of K^{42} were given intravenously to both subjects. At about 5 a.m. 300 grams of glucose dissolved in 1 pint of water was ingested by each. Thereafter, frequent blood samples were drawn for whole blood potassium, serum potassium and radioactive concentrations. (For technical reasons the whole blood determinations in this part of the experiment were considered unreliable and were discarded.) Urine collections were continued. Clinical evaluations and almost continuous K^{42} body readings in both patient and control were made for several hours. In addition, repeated electrocardiographic tracings were taken on the patient shortly after the glucose was given.

Shortly after the successful production of this second attack, the patient was fed liberal amounts of KCl and brought out of the attack.

In the afternoon of September 13, after the patient was relatively free of symptoms, an attempt was made to induce a third attack by water diuresis of potassium according to the method of Gammon (43). In the course of 3½ hours the patient drank over 4 quarts of water but no attack developed.

RESULTS

Two attempts to induce attacks of paralysis by ingestion of 300 grams of glucose were successful, the second attack being less profound than the first. The development of an attack was on each occasion associated with a sharp fall in the serum potassium concentration to a subnormal level (fig. 1); recovery in each instance followed the administration of large amounts of KCl and was accompanied by a rise in serum potassium level. In the first attack the serum potassium fell from a pre-induction 10:00 p.m. level of 3.7 milliequivalents per liter (14.7 mgm./1000 c.c.). The usual 10:00 p.m. level in the first 5 days of observation had varied between 3.7 and 3.9 milliequivalents per liter. It reached a low value of 2.5 milliequivalents per liter (9.9 mgm./100 c.c.) almost 17 hours later. At 15 minutes after the glucose a beginning fall to 3.5 milliequivalents per liter (13.8 mgm./100 c.c.) was already detectable, and by 2 hours it had dropped to 2.6 milliequivalents per liter (10.2 mgm./100 c.c.). Intermediate determinations on specimens taken between these two showed a continuous rapid decline in the serum potassium level. The usual preliminary 2:00 a.m. serum potassium levels had varied between 3.5 and 4.0 milliequivalents per liter. The value at 1:30 a.m., 3 hours after glucose ingestion was 2.6 milliequivalents per liter.

Blood glucose values in the meantime went from a pre-induction level of 61 milligrams per 100 cubic centimeters to 99 milligrams per 100 cubic centimeters at 15 minutes after glucose and reached a peak of 175 milligrams per 100 cubic centimeters at 1 hour after glucose. There was no significant abnormality in this curve (fig. 2).

In the second attack the same serum potassium pattern was repeated (fig. 1), the level beginning at 3.8 milliequivalents per liter (15.0 mgm./100 c.c.) before glucose, reaching 3.5 milliequivalents per liter (13.5 mgm./100 c.c.) at 15 minutes after and a low value of 3.1 milliequivalents per liter (12.1 mgm./100 c.c.) at 2 hours after the glucose. The serum potassium falls in both attacks were significantly greater than could be accounted for by the natural fluctuation of serum potassium levels which occurred during the relatively symptom-free preliminary 5 day period of observation. Although the severity of an attack in general paralleled the period of low serum potassium levels, an exact linear relationship between the two was not demonstrated. For example, at the start of the 7th day (A, fig. 1), the paralytic attack was deeper than at the height of the second attack (C, fig. 1), despite the fact that the serum potassium levels were comparable. Moreover, when the serum potassium levels were the lowest, the severity of the attack (B, fig. 1), was not significantly greater than at point A, when the serum potassium was 0.7 milliequivalents higher. Nevertheless, with minimum serum potassium levels there were, in general, maximum symptoms.

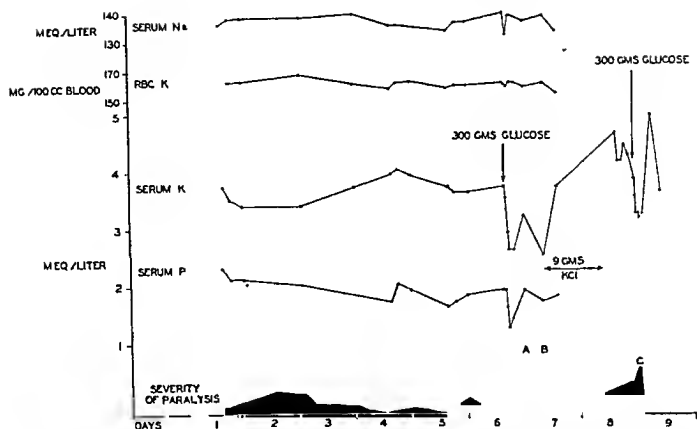


FIG. 1. ELECTROLYTIC DETERMINATIONS OF THE BLOOD AND THE CLINICAL CONDITION OF THE PATIENT DURING THE PRELIMINARY OBSERVATION PERIOD AND TWO ARTIFICIALLY INDUCED ATTACKS OF PARALYSIS

Significant changes associated with the development of an attack are evident in the serum potassium and serum phosphorus curves. Major attack followed administration of glucose in each case as denoted by the subsequent increase in the severity of paralysis.

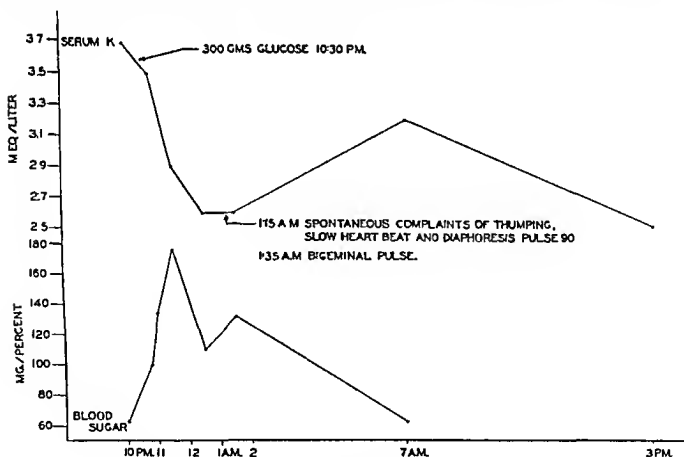


FIG. 2. SERUM POTASSIUM AND BLOOD GLUCOSE CONCENTRATIONS DURING THE FIRST INDUCED ATTACK

The control subject who received 300 grams of glucose in connection with the radioactive portion of the experiment under identical circumstances as the patient did not develop

symptoms despite the fact that his serum potassium fell from a level of 4.6 milliequivalents per liter (18.0 mgm./100 c.c.) before glucose to 3.4 milliequivalents per liter (13.4 mgm./100 c.c.) 11½ hours later. At 15 minutes after glucose his serum potassium was 4.1 milliequivalents per liter (16.0 mgm./100 c.c.) and at 2 hours it was 4.0 milliequivalents per liter (15.4 mgm./100 c.c.).

It is significant in this regard that in the second experiment, a bigeminal pulse rhythm was detected 8 minutes after the beginning and 5 minutes after the completion of the drinking of the glucose solution. This was verified electrocardiographically within 10 minutes of completing the glucose ingestion (fig. 3). Inverted T waves are also present in leads 2

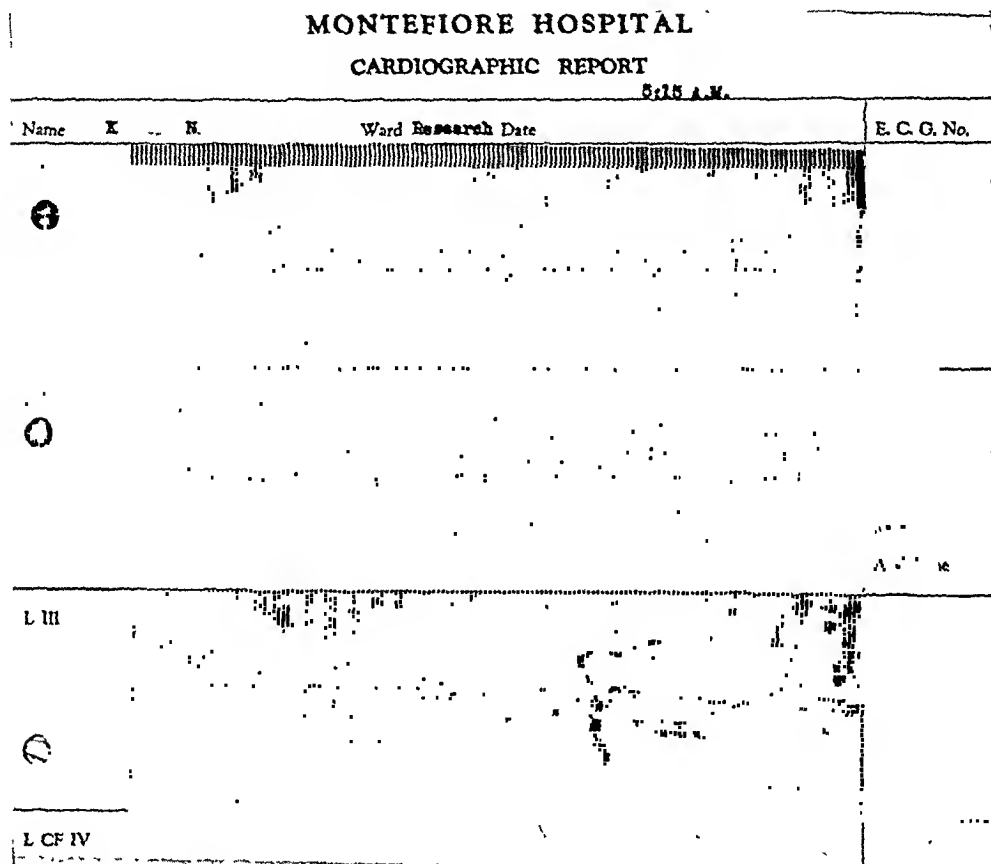


FIG. 3. ELECTROCARDIOGRAM TAKEN WITHIN 10 MINUTES AFTER INGESTION OF 300 GRAMS OF GLUCOSE FOR THE SECOND INDUCTION

The bigeminal rhythm is indistinguishable from that produced by digitalis toxicity. Inverted T waves are present in leads 2 and 3.

and 3 of this electrocardiogram. Subjectively, the first complaint noted in this recumbent, somnolent patient was "thumping, slow heart beat".

The red blood cells in 100 cubic centimeters of whole blood contained between 156.6 and 159.5 milligrams of potassium during one attack (fig. 1). There was no increase in the concentration of red blood cell potassium prior to or during this first attack. This value remained relatively constant throughout the period of observation.

The potassium output, which was almost entirely urinary, showed a marked fall during paralysis in the first induced attack (fig. 4). During the preliminary period the potassium output had varied between 2.89 and 3.98 grams per 24 hours. During the first day of paralysis, of which only 8½ hours belonged to the post-glucose period, the output for 24 hours was

1.55 grams. On the second day, despite the fact that the patient was fed several grams of KCl his output was only 0.67 grams. This coincided with a low urine output of only 660 c.c. On the next day, when the urinary output was over 2000 c.c., there was a slightly greater potassium output, 2.5 grams, although it did not reach the pre-attack levels.

When the potassium output was examined fractionally, it was apparent that during the first attack although there was a slight decrease in the urinary potassium output in the 7:00 p.m. to 11:00 p.m. and in the 11:00 p.m. to 7:00 a.m. portions, the most striking reduction was in the 7:00 a.m. to 7:00 p.m. fraction. This fraction on the day following the glucose when the patient was in a severe attack amounted to only 0.15 grams whereas usually he had excreted in his urine in the same period over 2 grams of potassium. Except on this day, 2/3 or more of the 24 hour urine output of potassium had occurred during this interval;

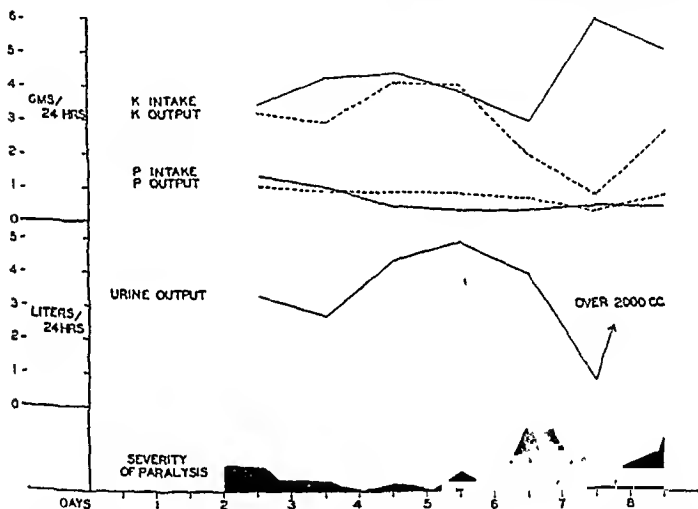


FIG. 4. INTAKE AND OUTPUT OF POTASSIUM AND PHOSPHORUS IN RELATION TO THE SEVERITY OF SYMPTOMS

There is a marked decrease in urinary output of both elements during the first induced attack.

On this day less than 1/3 of it occurred during this period. The daily potassium intake was never lower than 2.78 grams.

The serum phosphorus levels were determined only during the first attack (fig. 1). A significant drop in the serum phosphorus level occurred during the induction of this attack. The low level was less sustained than that of the potassium and also relatively less marked. The pre-induction value was 1.9 milliequivalents per liter (3.3 mgm./100 c.c.) and 15 minutes after the glucose it was the same. At one hour after glucose it was 1.6 milliequivalents per liter (2.7 mgm./100 c.c.) and at 2 hours it reached a low of 1.2 milliequivalents per liter (2.1 mgm./100 c.c.) By 8½ hours after the glucose, when the paralysis was fully developed, the serum phosphorus value had recovered to 1.9 milliequivalents per liter and remained at that level throughout the first attack.

There was a decrease in the urinary phosphorus output during the first day of the paralysis coinciding with the lowered potassium output of that day. The output (all urinary)

was only 0.20 grams compared to daily urinary outputs of between 0.78 to 1.01 grams on the pre-attack days. The decrease on this day was due almost entirely to a decrease in the 7:00 a.m. to 7:00 p.m. fraction. Usually more than 0.35 grams of phosphorus were excreted during this period whereas only 0.05 grams were recovered from the urine during the same period on this day when he was in a severe attack. The phosphorus intake had been relatively constant for 3 days prior to the attack.

Except for a drop in the serum sodium level from 139.0 milliequivalents per liter (320.0 mgm./100 c.c.) at 15 minutes after the glucose to 131.0 milliequivalents per liter (303 mgm./100 c.c.) 45 minutes later and recovery to 138.0 milliequivalents (317.0 mgm./100 c.c.) after another hour, there was no remarkable change in the serum sodium curve before or during the first attack (fig. 1). This low serum sodium curve coincided with the peak in the glucose curve and its recovery coincided with falling glucose levels.

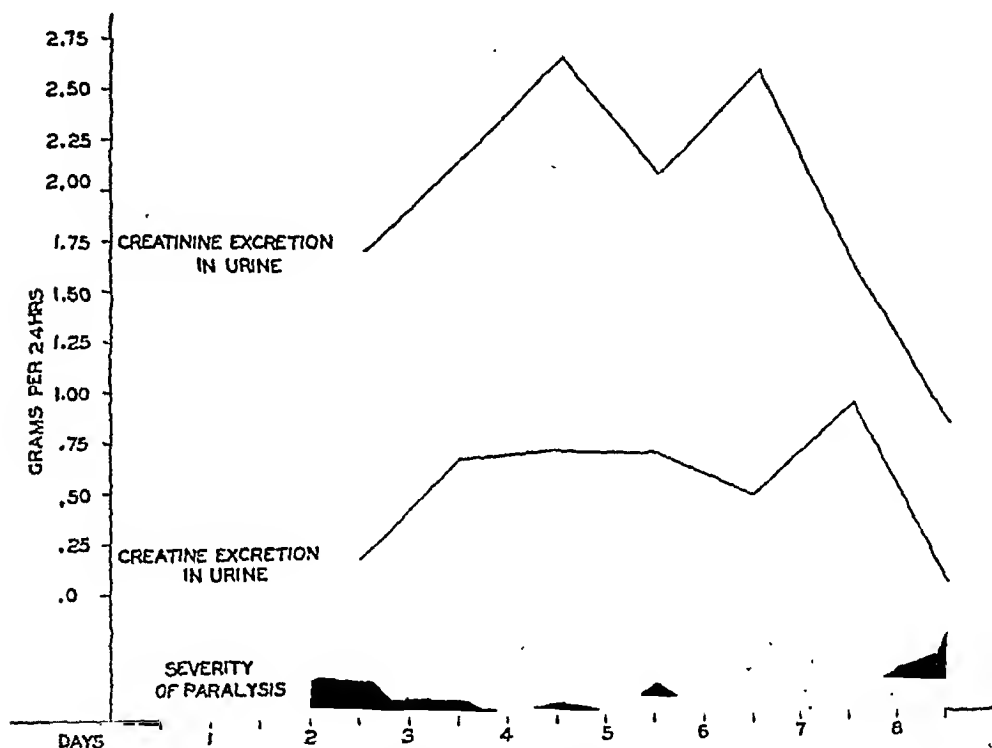


FIG. 5. URINARY EXCRETION OF CREATINE AND CREATININE

There is no apparent relationship between these abnormally high values and the presence or severity of symptoms.

A constant creatinuria was observed throughout the second admission of the patient and at times the creatinine excretion was abnormally high (fig. 5). Neither of these abnormalities bore any relationship to the presence or severity of symptoms.

The blood pressure showed remarkable fluctuations throughout the period of observation which could not be related in any rational way to the presence or absence of a paralytic state, nor to the level of the serum potassium. During attacks, the blood pressure varied from 66/48 to as high as 180/60 and 166/110. When well, readings varying from 80/40 to 170/122 were obtained. On one occasion when the patient was obviously emotionally tense the blood pressure was 166/118.

No significant alterations were noted in the blood counts and smears except for an eosinophilia, varying between 3 and 8 per cent, during his second admission. This could be accounted for by the presence of his hay fever.

The spinal fluid potassium during a symptom free period was 2.9 milliequivalents per liter (11.5 mgm./100 c.c.) and during a maximal attack it was 3.0 milliequivalents per liter (11.8 mgm./100 c.c.). Except for a spinal fluid protein of 60 milligrams per 100 cubic centimeters during the severe attack of his first admission, there were no other abnormalities noted in his spinal fluid examinations.

During attacks of paralysis Jolly reactions were noted in the parotid thigh muscles.

Alterations in the pulse during attack states consisted of the appearance of a bigeminal rhythm with a strong beat followed closely by a weak one. In the first induced attack this was observed 2½ hours after the glucose. In the second attack bigeminal rhythm was noted 8 minutes after the glucose drink was given.

In addition to the electrocardiographic record shown in figure 3 in which inverted T waves and bigeminal rhythm, with normal ventricular complexes followed by ventricular premature systoles, are demonstrated, other electrocardiograms taken during an attack show inverted T waves only, while similar records taken during well periods are normal.*

Other determinations revealed that during attack-free periods the basal metabolism, blood cholesterol, blood urea nitrogen, cephalin flocculation and thymol turbidity values were within normal limits. The serum total protein was 6.40 grams per 100 cubic centimeters with an albumin fraction of 4.60 grams and a globulin fraction of 1.80 grams per 100 cubic centimeters. The icteric index was 7. The 17-ketosteroid output measured 10.2 milligrams per day several days after recovery from the last attack.

The third induction attempted by forced drinking of water according to the method of water diuresis of Gammon, Austin, Blithe and Reid (43) was unsuccessful. Whether this failure was the result of the development in any degree of a refractory state following repeated attempts at inductions such as has been mentioned by Pudenz, McIntosh and McEachern (77), or to the ineffectiveness of the method in this patient was not definitely determined. It is noteworthy that the second induction did not produce as deep an attack as the first.

COMMENT

The results of our investigation indicate that attacks of paralysis in our patient with periodic paralysis may be induced by the ingestion of large amounts of glucose and that these attacks may be associated with definite electrolytic changes in the blood and urine. Such chemical changes have been previously recorded in the literature (1, 39, 43, 77, 89). The most evident of these was the fall in the serum potassium level coincident with the development of paralysis. This hypopotassemia was unaccompanied by increased potassium excretion in urine or stool. Not being related to potassium dietary deficiency nor to excessive excretion of potassium, it represents an intrinsic loss of potassium from the serum into other fluids or tissue compartments of the body. The consistency of these findings concerning the changes in the potassium metabolism should prove of diagnostic value.

In the search for loci where potassium could have accumulated after leaving the serum we were unable to demonstrate, during one of the attacks, any change in the concentration of potassium in the red blood cells (uncorrected for cell water changes). Talbott (89), also, did not find any such change in the red blood cells during paralysis. Pudenz, McIntosh and McEachern (77), however, in two attacks noted fluctuations in the red blood cell potassium content which they could not interpret.

* Electrocardiographic changes in this patient will be reported separately.

Attacks of paralysis associated with a lowered serum potassium have been produced in patients with periodic paralysis by injections of insulin. In normal individuals insulin causes a drop in serum potassium and it has been shown that the potassium disappearing from the serum does not enter the red blood cells (59, 60). It is unlikely therefore that potassium accumulates in red blood cells in periodic paralysis.

The spinal fluid potassium level in the lumbar sac was not significantly altered during paralysis despite simultaneously lowered serum potassium. The urinary excretion of potassium during such periods of maximal paralysis was significantly reduced. The renal threshold for potassium is directly correlated with its serum concentration (102). The rate of excretion decreases as the serum concentration is lowered so that the suppression of urinary potassium excretion during paralysis probably reflects a lowered level of serum potassium. These findings confirm previous investigations of both induced and spontaneous attacks.

Earlier reports have been inconsistent concerning the changes in the serum phosphorus levels associated with the development of an attack. Close observations on this patient during one attack confirm the presence of serum phosphorus level changes during paralysis. These parallel those of potassium though they are less marked or sustained. Moreover, as in the case of potassium, during the state of maximal paralysis there was a marked decrease in the urinary phosphorus output despite a relatively constant phosphorus intake.

We were unable to demonstrate any significant change in the serum sodium level during the development of an attack except for a transient fall immediately following the ingestion of the glucose solution. This may only have been due to an osmotic reaction to the sudden intake of a large amount of hypertonic glucose into the intestine. Ferrebee, Gerity, Atchley and Loeb (39) showed no change in serum sodium in one attack and demonstrated a normal relation of sodium balance to water balance and extracellular fluid volume. Urinary sodium excretion was not remarkable in their patient.

No abnormalities of the glucose tolerance of our patient were noted.

There was no convincing clinical evidence of hepatic, thyroid or adrenal dysfunction when the patient was well.

Abnormal creatine-creatinine metabolism with excessive excretion of both compounds, such as was evident in our patient, has been repeatedly noted by other observers. Inasmuch as muscle creatine appears to be the precursor of urinary creatinine (8), these findings merely reflect a disordered muscle function.

Certain observations of incidental interest were noted in the radioactive experiment which appeared reliable and should be recorded. The urinary excretion of K^{42} of the patient was about twice that of the control. In 39 hours following K^{42} injection the patient excreted more than 7.9 per cent of the original dose whereas in the same period the control excreted only 4.5 per cent of a similar original dose. There has been no opportunity to verify this observation. If verified it might be very significant. One explanation might be that the kidney in patients with periodic paralysis is unable to retain added potassium as well as in normals. A less likely explanation would be that these patients do not handle K^{42} in the same fashion as ordinary potassium. Both subjects showed a con-

stantly increasing blood K^{42} concentration beginning about a half hour after the intravenous K^{42} injection. This seems paradoxical but nevertheless is confirmatory of work on animals done by Joseph, Cohn and Greenberg (56), Wilde (98) and Fenn (33). Soon after injection they found that the marked potassium reached a high concentration in the liver, after which it slowly moved out of the liver, and the concentration in the muscles and in the whole blood increased slowly. A third observation, for which no explanation is apparent, was that surface readings of K^{42} on the Geiger-Mueller counter over the head (mastoid area) on both subjects were much higher than those over liver or muscle areas in the first few hours after K^{42} injection. These higher values, moreover, were probably not due to greater scalp vascularity inasmuch as with decreasing head values as time progressed, actual blood K^{42} concentrations by direct measurement were increasing. The tracer technique used during the induction of an attack failed to reveal any change in marked potassium concentration in the tissues measured.

Potassium in Relation to Metabolism of Carbohydrate, Muscle and Nerve

Most of the body potassium is located in the muscles where its concentration is about 30 times that in the tissue spaces (35, 45). It is the chief intracellular cation of the organism, capable of passing through tissue membranes with ease. Its high intracellular concentration is maintained in part, at least, by the needs of intracellular, impermeable anions for an available, easily diffusible cation to satisfy the requirements of osmotic and electrical equilibrium (34, 80, 101). The evidence supporting a secretory function of the cell membranes controlling the permeability of potassium (101) is not strong. Under circumstances which deplete body stores of potassium, such as after administration of desoxycorticosterone or after a potassium deficient diet, intracellular potassium may be replaced by sodium (40, 49, 71), and possibly even by the ammonium ion to some extent (10, 38). Magnesium, the only other cation present within the cells in any large amount, may also adjust its concentration slightly in response to changes of intracellular potassium in an effort to maintain equilibrium within the cell (10, 13, 49). Such replacements or adjustments may occur within physiological limits without disturbing the intracellular osmotic relationships or the ability of the cell to contract (19, 34).

Ordinarily concentration gradients exist between the intracellular potassium content and that of the interstitial fluid and to a lesser extent between the latter and that of the serum. Thus, if the serum potassium level falls sufficiently that of the interstitial fluid follows and the intracellular content of potassium in turn may respond within its limitations (13, 34, 68, 101). Changes of intracellular electrolytic balance thus produced are believed to be brought into equilibrium by shifts of cellular water or by the entrance of other cations (chiefly sodium) into the cell (28, 30, 103). Such gradients, however, do not always exert inflexible rigid control over the potassium content within the cell and it has been shown that normal muscle cell contents may sometimes exist with low serum values and vice versa (70, 103).

There is increasing evidence that potassium plays an essential role in carbo-

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We were unable to demonstrate any significant change in serum sodium level during the development of an attack except for a transient increase following the ingestion of the glucose solution. This is probably an osmotic reaction to the sudden intake of a large amount of glucose into the intestine. Ferrebee, Gerity, Atchley and others have shown that in serum sodium in one attack and demonstrated a balance to water balance and extracellular fluid balance. Urinary excretion was not remarkable in their patients.

No abnormalities of the glucose tolerance test were observed.

There was no convincing clinical evidence of impairment of renal function when the patient was well.

Abnormal creatine-creatinine metabolism was observed in compounds, such as was evident in other observers. Inasmuch as most of the urinary creatinine (8), these findings are consistent with the findings of other observers.

Certain observations of incidence of periodic paralysis which appeared reliable in the case of K^{42} of the patient was abnormal. Following K^{42} injection the patient showed a decrease in excretion whereas in the same period of time on the original dose. There has been no verification of it might be verified in patients with periodic paralysis in normals. A less marked decrease in K^{42} in the same fasting state.

leave the cell, and when they are reformed, the reverse should occur. Such large variations in normal muscle potassium, presumably due to such behaviour, have been reported although there is some disagreement on this point (47). This has prompted some workers (13) to divide the intracellular potassium into two types, a "basal" portion, which is held within the cells in a basal static condition, and a "metabolic" portion, which represents that which enters the cells to meet the changing temporary demands of carbohydrate metabolism. The increase of lactic acid and potassium in the blood after muscular activity, which is associated with breakdown of glycogen, also supports this hypothesis.

Glycogen in muscle serves in part, at least, as a source of energy for the generation of certain energy-rich phosphate organic compounds among which are phosphocreatine, adenosinetriphosphate, acetyl phosphate and phosphopyruvate, important to the so-called adenylic system. When these high energy compounds react with certain intermediary substances or are hydrolyzed to inorganic phosphate, energy is released which is essential either directly or indirectly for muscular contraction (5, 85). It has been demonstrated that certain intermediate steps in the resynthesis of some of these high energy compounds cannot take place in the absence of potassium. Neither phosphorylation of the adenylic acid system for the resynthesis of adenosinetriphosphate, nor rephosphorylation of creatine can take place without the specific presence of this element (9, 10). It has been noted that the creatine and potassium contents of voluntary muscle vary directly, both increasing or decreasing together, in the ratio of 1 mole of creatine to 2 of potassium suggesting that phosphocreatine is present within the cell as a dipotassium salt (74). There is also experimental evidence that adenosinetriphosphate and other organic phosphorus compounds exist within the cell as potassium salts (73).

Abundant evidence is available indicating the importance of potassium for the normal functioning of nervous tissue. The energy rich carbohydrates and organic phosphates exist and function in nervous tissue much as they do in muscle tissue, and the dependence of them upon potassium can be assumed. There is some evidence in support of this (7, 92). Potassium, also, is known to play an important role in the release of acetylcholine and has been found essential for the resynthesis of choline acetylase, an enzyme essential for the synthesis of acetylcholine (7, 34, 75). Its importance in the transmission of the electrical impulse along a nerve fiber or across the myoneural junction is generally recognized.

There is some difference of opinion as to the mechanism of action involved in the lowering of serum phosphate and the raising of muscle hexosemonophosphate after insulin and after adrenalin (58). Some believe that the increase in muscle hexosemonophosphate results from the esterification of inorganic phosphate leaving the blood to enter the muscle, and that this process is an adrenalin response, the similar effect following insulin resulting from a reflex stimulation of adrenalin consequent to insulin hypoglycemia (18). Others maintain that the decreased serum phosphate concentration can be accounted for by a rise in liver phosphate, suggesting that liver esterification of phosphate is important

(57, 76). A third viewpoint is that the fall in blood inorganic phosphate is an insulin response due to esterification of the inorganic phosphate outside the muscle, whereas the rise in muscle hexosemonophosphate due to adrenalin, results from the breakdown of glycogen, and that each hormone evokes the secretion of the other (84). The concomitant lowering of serum potassium which also occurs after insulin or adrenalin can be explained in each hypothesis by the apparent need for potassium by phosphorylation of either glucose or glycogen. There is slight evidence that the potassium content of muscle is increased after adrenalin (34).

In adrenal cortical insufficiency, as in Addison's disease and in adrenalectomized animals, the serum and muscle sodium contents are decreased as sodium is lost from the body. At the same time, due to impaired kidney excretion of potassium and leakage from the tissue cells into the extracellular fluid, the serum potassium level rises. The accumulation of potassium is also reflected in a rise in muscle potassium content (although liver potassium is not raised). These effects can be overcome by replacement of adrenal cortical hormone which allows a greater renal excretion of potassium with consequent reduction of serum and muscle potassium levels to normal and retention of sodium in the body. If too much adrenal cortical hormone is given (or in potassium deficient animals), the serum and muscle potassium contents may fall below normal; sodium then replaces potassium lost from the cell, and if this is allowed to go uncorrected, paralysis may occur which can be corrected by administering potassium. Interference with cardiac function may similarly develop, with evidence of impairment of auriculo-ventricular conduction, which may also be corrected by potassium. It is recognized that not only potassium administration, but carbohydrate, as well, may precipitate a crisis in patients with Addison's disease, presumably due to further increase in intracellular potassium necessary to metabolize the carbohydrate. It has been suggested that desoxycorticosterone acts by affecting membrane permeability (5, 13, 27, 34, 40, 47, 61, 91).

DISCUSSION

Attacks of periodic paralysis frequently follow ingestions of large amounts of carbohydrate. Symptoms of an impending attack may often be aborted by the patient "walking it off." Such activity, by the effects of muscular contractions, may be presumed to release potassium from its combination with high-energy phosphate carbohydrate esters as the latter are reduced to lactic acid thus making available an extra source of potassium. Periodic paralysis occurring in patients with hyperthyroidism has been reported on several occasions (51). Whether this concurrence is based upon excessive carbohydrate intake secondary to increased metabolic needs in patients with a latent tendency towards the disease, or upon some more fundamental endocrinological disorder of carbohydrate metabolism is unknown.

Experimentally, attacks of paralysis associated with a low serum potassium have been produced in patients with periodic paralysis by large amounts of oral glucose or heavy carbohydrate meals; by administrations of levulose; by insulin

injections alone, or more easily, in combination with glucose; by injections of adrenalin; by water diuresis of potassium following forced drinking; and by injections of adrenal cortical hormones (1, 2, 39, 43, 77, 78, 81, 89). In the normal individual, most of these procedures, with the quantities given, will produce a fall in the serum potassium level without the development of paralysis (1, 2, 16). In general, as in our control, the fall is neither so low nor so rapid. The actual level of serum potassium does not seem to be a crucial point in the development of paralysis for levels as low as 7.2, 8.5 and 9.4 milligrams per 100 cubic centimeters in diabetics after insulin (1) and as low as 10.5 milligrams per 100 cubic centimeters in normals after adrenalin (2) have been produced without causing any paralytic symptoms. Moreover, attacks of paralysis with normal serum potassium levels have been reported in patients with periodic paralysis (3, 26, 77, 95). Our failure to find an exact linear relationship between the depth of attack and the serum potassium level supports the contention that the actual serum potassium level does not cause the paralysis. It is possible that in the patients who had normal serum potassium levels during paralysis, had more frequent specimens been taken, some of these may have disclosed low potassium contents; but, in any case, these normal serum values do not necessarily indicate the physiologic state within the cell. It may be assumed that it is the cellular potassium content which controls the threshold determining the appearance of paralysis in these patients.

Dogs dying in paralysis from overdose of desoxycorticosterone have revealed in their skeletal muscles large amounts of intracellular sodium and less than normal amounts of intracellular potassium (40). Similar changes have been reported for rats (40). In humans overdoses of desoxycorticosterone have caused paralysis associated with large losses of potassium from the body.

Based upon the validity of this assumption, the suggestion appears plausible that the chief metabolic differences between the response of the susceptible patient and the normal individual to potassium lowering stimuli are in the concentrations of the muscle cell potassium and the rates of fall of the serum potassium level, both of which may be functions of the same metabolic defect. The importance of the rate of change of potassium concentrations has been pointed out in other functions. For example, in the development of heart poisoning by potassium increases, it appears that the rate at which the potassium rises in the serum and presumably the rate of its penetration of cells in the heart is an important factor in the development of toxicity (19).

Transient muscular paralyses with electrocardiographic abnormalities have been reported in chronic nephritis (12) and in poorly controlled diabetes (52) where low serum potassium levels were found. A response to potassium therapy was obtained in these cases. No determinations of cellular potassium nor of the rates of change of serum potassium levels were made in these instances.

Not all the evidence, however, is consistent with the viewpoint that the interference with normal potassium physiology is due to changes in its intracellular content. From the effects of potassium toxicity due to hypernormal rather than subnormal levels, there is some indication that the extracellular fluid potassium

content is the important factor (19, 29, 70). Thus, it has been found that potassium poisoning and resultant muscular dysfunction may occur in animals where the added potassium is reflected in an elevated serum potassium level while the skeletal muscle potassium level remains low. Moreover, the toxic effects of injected potassium have been shown to be directly related to the elevation in concentration of serum potassium and only indirectly to that of muscle potassium (70). It should be borne in mind, however, that this is evidence based on potassium toxicity rather than potassium deficiency, and it is, perhaps, not valid to draw conclusions from this as to the mechanism of periodic paralysis.

Miller and Darrow (69) have failed to alter muscle contractility in rats by changes either in muscle potassium or by low concentrations of serum potassium. This has prompted them to state that disturbances in muscle potassium are not *per se* the explanation of familial periodic paralysis. Their findings are not in agreement, however, with repeated observations of others (35, 48). A point of difference in the experimental techniques of Miller and Darrow and others which may be significant is that the potassium deficiency produced by them, although profound, was achieved slowly and gradually by potassium deficient diets and by adrenalectomy, whereas others have produced rapid changes of potassium by perfusion techniques and have succeeded thereby in altering muscle activity (69). Here, again, the rate of potassium change appears to be important.

Transient paralysis and cardiac arrest have been reported in 2 cases of high serum potassium toxicity by Finch and Marchand (41), in one of which a subsiding paralysis returned following a dose of KCl. In each instance, a flaccid paralysis developed, sparing the head, and was characterized by areflexia and intact mental and sensory functions, findings observed in periodic paralysis. The issue is raised, therefore, whether or not the potassium disappearing from the serum in periodic paralysis may have produced an intracellular potassium poisoning in neuro-muscular tissue. Such a concept has been suggested by Serebryanik and Zaroquentseva (81). This seems to be unlikely, for not only would the potassium then be going against the gradients, but one would not expect a therapeutic effect from potassium administration. Moreover, others have successfully produced attacks of paralysis in susceptible patients by each of two methods which act by depleting body potassium, namely, washing potassium from the serum and tissues by water diuresis and by administration of desoxycorticosterone. In addition, electrocardiographic changes when present in familial periodic paralysis show low T waves (87, 88) rather than elevated T waves as occur in potassium toxicity (41, 90, 99).

Of special interest and significance is the rapidity with which cardiac disorder developed following the ingestion of glucose in the second induction of our experiment. Within 5 minutes after the completion, or within 8 minutes of the beginning of the ingestion of 300 grams of glucose dissolved in 450 c.c. of water, the patient developed bigeminal rhythm which was confirmed electrocardiographically. That this cardiac effect was not caused by vagal stimulation as a result of gastric distention is supported by the following facts. In the first experimental induction of paralysis an abnormal bigeminal radial pulse rhythm, was

noted $2\frac{1}{4}$ hours following the ingestion of a similar quantity of fluid and persisted for at least an hour thereafter. The abnormal pulse in the second induction also persisted for at least an hour. The delayed onset of arrhythmia in the first instance, and the persistence of both for prolonged intervals do not seem consistent with the effects of vagal stimulation from gastric distention. Moreover, at the end of the experimental procedure an attempt was made to induce an attack of paralysis by forced drinking and during a period of $3\frac{1}{4}$ hours the patient drank over 4 quarts of water, often several glasses in succession, without the development of any disorder.

It has been noted experimentally that insulin may stimulate the vagal centers (5), but the rapidity of onset of arrhythmia in the second attack is not compatible chronologically with sufficient insulin response to the glucose ingestion for such an effect.

Although bigeminal rhythm has not heretofore been described in familial periodic paralysis, delayed auriculo-ventricular conduction has been noted in this disease as well as in hypopotassemia in other conditions such as desoxycorticosterone acetate toxicity and chronic nephritis (12, 87, 88). The finding in this case, however, of bigeminal rhythm is to be compared with the presence of the same findings in digitalis toxicity from which it is indistinguishable. There is considerable evidence pointing to the fact that a cardiac intracellular potassium deficiency is produced in overdoses of digitalis (15, 96). Moreover, it is known that premature systoles in such toxicity may be dispelled by the administration of potassium salts (80). That the bigeminal rhythm is an hypopotassemia affect is further supported by the associated presence of depressed and inverted T waves in the electrocardiogram.

Pudenz and his co-workers (77) noted that the bradycardia of 40 in their patient increased to 120 after atropine, and Stoll and Nisewitz (88) observed that the electrocardiographic changes (delayed conduction) in their patient with periodic paralysis were also corrected after atropine, suggesting that the hypopotassemia produces its cardiac effects by a vagal-like action. That potassium concentrations are intimately involved in the activity of the vagus nerve is well known, but the stimulating effect upon the vagus is produced by increases of potassium levels, rather than the lowered potassium levels presumed to be in effect here. The atropine used by these workers may have suppressed normal vagal tone, thus improving impaired conduction from another cause, perhaps a direct effect of potassium deficiency upon the conduction system distal to the vagal endings. The findings of low T waves rather than high ones, as stated above, refutes the contention that intracellular cardiac potassium is elevated in periodic paralysis.

That such a rapid development of cardiac dysfunction as occurred in our experiment could have developed as a result of rapid shifts of potassium is further supported by the findings on both occasions of a decreasing serum potassium level already detectable on the first post-glucose specimens drawn 15 minutes after the glucose was taken. Castleden (16) has shown that after adrenalin is injected intramuscularly in normal subjects, the serum potassium begins to fall

at the end of 3 minutes, and that most of the fall occurs within 10 to 20 minutes. It is known from other sources that movements of potassium may be very rapid. Its entrance into the liver occurs with great rapidity presumably because it enters in combination with some anion as an isotonic solution rather than by the slower process of cation exchange such as occurs in muscle (34). Thus, in cats, within 15 to 25 minutes, injected radioactive potassium reaches a minimal value in the plasma, and a large portion of it has entered the liver cells (33, 98). After this there is a secondary rise of marked potassium in the blood (detected in the radioactive part of our study) reaching a maximum 40 minutes after injection.

There are two possible mechanisms which may have been responsible for producing a strong intrinsic demand for potassium immediately following the ingestion of glucose. The first and probably the most important is the liver. The absorption of glucose, which occurs primarily in the small intestine, is rapid and rises in blood sugar following its ingestion have been shown in animals to be detectable within 2 to 3 minutes (5). It has usually been thought that the rate of absorption of glucose from the intestine has been limited to a maximal amount of 0.85 grams per kilo per hour, regardless of the quantity available in the intestine (97). However, under conditions of forced feeding with strong glucose solutions, as in this experiment, maximal absorption rates are present only at the beginning of absorption and decrease thereafter with time (64). Thus, within a few minutes following the beginning of glucose ingestion, this sugar can be assumed to have been reaching the liver via the portal system at a maximal rate. Venous hyperglycemia has not yet developed, however, so that the glucose concentration in the systemic circulation is not yet at a maximal value. Most alimentary glucose undergoes glycogenesis rapidly in the liver, and, as has already been pointed out, this process demands the presence of a definite amount of potassium. Thus, maximal hepatic glycogenetic drain on potassium within a short period of time, as evidently occurred in this patient, is understandable.

The second possible site where potassium may have been in immediate demand is the intestinal mucosa. There is experimental indication that glucose absorption through intestinal mucosa is associated with phosphorylation of the glucose (5). This is based in part upon the fact that phosphorus content of intestinal mucosa increases during glucose absorption (31). As we have seen, the process of phosphorylation probably depends upon the presence of potassium, so that it may be justifiable to suggest that the potassium content of intestinal mucosa may also increase during glucose absorption. It has been shown in frogs that potassium does increase the permeability of the intestinal wall to glucose (44).

Thus, mobilization of potassium by hepatic glycogenesis and intestinal glucose absorption could account for the cardiac arrhythmia in this patient. This cardiac disorder was probably related to a falling potassium content of cardiac muscle. That the heart alone and not the other tissues was so readily affected by potassium deficiency is not surprising inasmuch as the reactions to potassium changes of various tissues have been shown to differ (27, 100). It is important

to remember that it may have been the rate of change of cardiac potassium, with uncompensated disturbances of potassium-calcium or other electrolytic balance, rather than the magnitude of cardiac potassium change which determined impairment of cardiac function.

For heart function to have been so quickly affected sufficient potassium must have been drawn out of the serum into the liver or intestinal mucosa creating a drain on cardiac muscle potassium within 5 to 8 minutes. If the withdrawal of such a quantity of potassium in that short a time is not physiological, this then may be the defect in potassium behaviour in periodic paralysis, namely, a pathological mobility of the potassium ion.

Extra-hepatic glycogenesis, likewise, may create a drain on body potassium, but at least at the beginning of a glucose induced attack this is probably of secondary importance. Subsequently, glycogenesis in muscle tissue, may play a rôle in creating this great potassium demand. Under such circumstances, if one places the locus of potassium deficiency which creates paralysis in muscle rather than in nerve tissue, it is necessary to postulate that the intermediate phosphorylating steps of glycogenesis bind potassium within the muscle cell to such an extent that there is not a sufficient amount of the element available for the intracellular potassium functions of the high energy phosphate systems in muscle.

Some indirect evidence, however, that muscle is not the site of excessive accumulation of phosphorylating carbohydrate compounds in this disease is derived from the observation of Brand and Harris (11). They found a decreased content of both creatine and acid soluble phosphate in the muscle of a patient with familial periodic paralysis. As noted earlier, parallel shifts of intramuscular creatine and potassium have been observed.

The absence in patients with periodic paralysis of the normal limitations on the glycogenetic drain on potassium may be explained by one of the following concepts: by greater amounts of potassium required in patients with periodic paralysis for glycogenesis in liver or other tissues; by some unnaturally maintained intracellular potassium deficiency in hepatic or other tissues; or by differences in the cellular permeability of certain tissues to potassium or to other cations capable of replacing potassium within the cell.

The clinical evidence for a central nervous system mechanism causing the paralysis in this disease stems chiefly from the observations of Pudenz, McIntosh and McEachern (77) who constricted one arm of a paralyzed patient above the systolic level, and then, after injecting 1 gram of KCl intravenously in the other arm, noted, within 5 minutes, recovery in all parts including the constricted arm. This was not confirmed, however, by Ferrebee, Gerity, Atchley and Loeb (39).

The clinical characteristics of the syndrome contradict the conception of a central neural etiology. The depression or absence of electrical irritability of muscle is strong evidence against it. This is supported by the failure to find changes in the cerebrospinal fluid potassium values by us and others (77, 89). Neurologically, the defect producing flaccid, areflexic paralysis without sensory change must be along a final common pathway beginning with the anterior horn cell and extending to the motor effector cell. It is very unlikely that potas-

sium deficiency affects the anterior horn cell selectively within the central nervous system.

Moreover, the validity of the experimental procedure of Pudenz and co-workers is questioned by the fact that the anoxic trauma of arterial constriction releases potassium from tissue cells (83). Thus, McCance and Widdowson (65) and Rewell (79) have reported increases in serum potassium in orthopedic operations following prolonged arterial constriction. In one subject of ours whose arm was arterially constricted from 25 to 30 minutes, the serum potassium below the level of constriction rose on one occasion from a value of 16.0 to 28.0 milligrams per 100 cubic centimeters, on another from 14.3 to 74.0 milligrams per 100 cubic centimeters, and on a third occasion, with the same technique, hemolysis with gross increase of potassium was evident after constriction.

It is not impossible that the peripheral conduction of the nervous impulse as well as muscular contractions may be affected by a severe serum or cellular potassium deficiency in periodic paralysis, but if this is so, it must be assumed that only spinal motor nerves are so affected inasmuch as sensory functions and usually cranial nerves are intact in this disease.

Confirmation of the potassium-carbohydrate relationship might be obtained from adequate potassium studies, which have not been reported, in von Gierke's disease, where abnormal accumulations of glycogen occur especially in the liver, kidneys and the heart. Low serum inorganic phosphate levels and flaccid, hypotonic musculature have been noted occasionally in this syndrome (93).

The rôle of potassium in the production of muscular weakness and paralysis raises the issue of its place in the etiology of muscular dystrophies. Several authors (6, 78) have noted the occasional incidence of patients with periodic paralysis developing progressive muscular dystrophic disease. Cummings has observed in muscle biopsy studies (20, 21, 22, 23, 24, 25) that the muscle potassium content was normal in most neurological conditions, except for three disease groups. Low values were found in neuromuscular dystrophies where normal muscle tissue was replaced by fat or connective tissue. Very high values were present in the involved muscles in myasthenia gravis and very low levels in the myotonias. Following prostigmine injections in the latter two conditions, the muscle potassium approached normal, decreasing in myasthenia gravis and increasing in myotonic conditions. In myasthenia gravis associated with a low concentration of muscle potassium following prostigmine, there was a concomitant increase in serum potassium with clinical improvement. When the effect of prostigmine wore off the muscle potassium rose again. The benefit clinically of potassium administration, in most cases of myasthenia gravis is generally accepted. This has been convincingly reported by Laurent and Walther (63). In myasthenia gravis, therefore, the extracellular potassium level may be of some etiological importance. Prostigmine in normals did not affect serum or muscle potassium levels. Following exercise in two patients with marked myotonia and minimal atrophy, clinical improvement was associated with a decrease in the serum potassium level. Muscle potassium concentrations were not determined, so that one can only guess that the muscle potas-

sium may have increased to normal. Glucose and insulin administrations in two patients with dystrophica myotonica failed to alter significantly the serum potassium level. In one patient with familial periodic paralysis, when the patient was well, a normal muscle potassium was found. Unfortunately, no determinations could be obtained during an attack. In one patient included in Cummings' reports who had muscular weakness associated with a parathyroid tumor, before removal of the tumor there was a low muscle potassium content, which decreased with prostigmine, and following removal, when the patient was recovered, there was a normal muscle potassium value which did not change after prostigmine. In nutritional muscular dystrophies produced in rabbits, low muscle potassium, creatine and magnesium values were obtained by Fenn and Goettsch (36), which were presumably proportional to the loss in numbers of intact fibers noted histologically. It is of further interest that we recently saw a ten month old female infant (L. F., M.H. #42112) with amytonia congenita who had persistently low serum potassium values.

What significance such potassium changes may have in the pathogenesis of dystrophic muscular disease remains to be determined. The incidence of endocrinological disorders in muscular dystrophies and the response of potassium to various endocrine stimuli strengthens the possibility of the etiological importance of potassium. Judging from the complexity of potassium behaviour it is unlikely that a single explanation of its disordered metabolism in any of these diseases will be applicable to all the others, nor possibly, even, to all cases of the same clinical syndrome.

It has been suggested by Jiménez-Díaz and his co-workers (54) on the basis of two of their own cases with suggestive hypopotassemia that abortive syndromes of periodic paralysis may account for occasional instances of recurrent marked asthenia and fatigability without demonstrable paresis.

In summary, the pathogenesis of periodic paralysis is based upon a metabolic disorder of potassium. The affection is often activated clinically by excessive carbohydrate metabolism. It seems likely, as Talbott (89) previously speculated, that the resulting hepatic glycogenesis is an important factor in creating a drain on body stores of potassium. The rapid onset of cardiac arrhythmia following glucose ingestion in this patient coincides in time with such an expected maximal concentration of potassium in the liver and so seems to bear out Talbott's hypothesis. In this disease, such a potassium mobilization exceeds normal limits. This defect must depend upon an unexplained natural deficiency of potassium in the liver or other tissues, upon an unexplained increased requirement for potassium in certain aspects of carbohydrate metabolism, or upon a change in cellular permeability to potassium or other cations. Any of these defects may be hereditary. The contractile high energy phosphate systems of the muscles are thus deprived of ample amounts of potassium needed for normal function. The potassium presumably remains unavailable until it is replaced from extrinsic sources or until normal carbohydrate utilization is sufficient to release ample amounts of potassium.

To further elucidate this concept it is suggested that in subsequent investiga-

tions controlled hepatic vein catheterization experiments be done for potassium and phosphorus contents of hepatic venous blood before and during the development of attacks. Simultaneous liver and muscle biopsies for potassium content should prove valuable. By determining in patients and controls the rates at which equilibrium is reached between K^{42} and ordinary potassium in various tissues and body fluids, further evidence of the possible rôle of pathological mobility of potassium in these patients may be found.

SUMMARY

1. Attacks of paralysis were induced on each of two occasions in a patient with periodic paralysis by the ingestion of 300 grams of glucose in hypertonic solution.

2. The development of these attacks was accompanied by falling serum potassium levels and was unassociated with concomitant excessive potassium excretion. Recovery was associated with a rising serum potassium level.

3. A comparable but less marked and less sustained fall and rise in the serum phosphorus level was noted in one closely observed attack.

4. A suppression of both phosphorus and potassium urinary excretion during the phase of maximal paralysis was noted.

5. An increased urinary excretion of creatine and creatinine was observed.

6. No significant changes were noted in the serum sodium levels, red blood cell potassium concentrations, spinal fluid potassium levels or the glucose tolerance of this patient in relation to the development of an attack of paralysis.

7. Pertinent current knowledge concerning normal potassium behavior as it is involved in carbohydrate, muscle and nerve metabolism is briefly reviewed.

8. Evidence is presented which supports the hypothesis that hepatic intermediate carbohydrate metabolism plays an important rôle in periodic paralysis by lowering potassium content of muscle.

9. That serum levels of potassium in periodic paralysis do not directly determine the onset of paralytic manifestations is emphasized.

10. Bigeminal rhythm in an induced attack of periodic paralysis is noted.

11. Incidental observations concerning K^{42} metabolism in humans are reported.

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AORTIC STENOSIS: A STUDY OF THE CLINICAL AND PATHOLOGIC ASPECTS OF 107 PROVED CASES*

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"To understand stenosis we must study it in its unmixed form."—ALBUTT.

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I. INTRODUCTION

Textbook discussion of aortic stenosis implies a degree of regularity and a conformity to pattern which might indicate that further study is superfluous. Recent experience on the medical wards where in a single week two patients with advanced calcific stenosis of the aortic valve died without our making the diagnosis prompted us to carry out the investigation we are now reporting. Although several methods suggest themselves, a study based on anything but autopsy reports leaves suspect the error of missed diagnosis and of wrong diagnosis. In order that the effect of disease of other valves be excluded in evaluating the clinical and morphologic findings, only cases in which stenosis of the aortic valve was the single significant valvular lesion were included. This selection permits us to relate symptoms and signs to lesions with more accuracy than in the presence of disease of one or more additional valves. Dynamic alterations such as mitral regurgitation may well have existed in some cases but aortic valve deformity was the only organic valvular lesion. The method of selection prevents us from bringing decisive evidence to bear on the moot question of pathogenesis since we excluded any case with mitral valve lesion, thus eliminating many instances of obvious rheumatic heart disease.

II. COMMENT ON THE LITERATURE

We have studied reports dealing with aortic stenosis in medical periodicals and textbooks (1-87). For the most part they do not supply a satisfactory basis of comparison with our material because they adhere too closely to a classical picture of the disease in dealing with clinical cases, or discuss but few cases, or combine clinical and post mortem material, or do not discriminate between isolated aortic stenosis and that complicated by mitral or other valve disease. While other valves are commonly diseased in association with aortic stenosis, and such combined disease of valves deserves study, our material was selected to exclude cases thus complicated. The problem of aortic stenosis *per se* is under consideration.

Recognition of aortic stenosis at the post mortem table is several centuries old (11, 59). It cannot be stated with certainty who was the first clinician to diagnose it at the bedside. By the end of the last century it was a commonplace diagnosis (12, 16, 26, 35, 41, 53, 63, 77), and was made on the basis of a systolic murmur heard best over the aortic area. After experience with effort syndrome in World War I the systolic murmur fell into disrepute; the diagnosis of aortic stenosis was made less often and interest in it declined. In the late

20's and early 30's the disease came back into prominence following the excellent papers of Christian (17, 18), McGinn and White (57), Marvin and Sullivan (55), Contratto and Levine (21, 22) and Dry and Willius (28, 29). A number of other cardiologists helped clarify our understanding of the disease (1, 3, 8, 9, 10, 13, 14, 19, 20, 34, 37, 43, 46, 51, 54, 65, 68, 70, 73, 75). To the basal systolic murmur transmitted into the neck, the thrill, the large heart and small, slow pulse were added once more syncope, cardiac pain and the risk of sudden death—all having been noted in early records of the disease (12, 35, 41, 53, 49, 63, 72, 77, 81), but not properly stressed in the problem of diagnosis. There are to be sure excellent discussions of the disease in some texts, notably Seherf and Boyd (67), but in many the oversimplification has given a distorted picture of the disease, or the data have been obtained from complicated cases (16, 21).

III. SELECTIONS OF CASES

The basis of this study consists of 107 autopsied cases selected from 15,016 consecutive records in the department of pathology in this hospital from the year 1924 through 1946. The portion of all records dealing with the heart was read in order to avoid omissions. The records include those from the pediatric service, and since the year 1930, from the Chronic Disease Hospital. These are all the routine autopsy reports compiled by senior and junior pathologists under the direction of Dr. Richard S. Austin.

Criteria for Selection and Classification: Cases were selected for analysis on the basis of finding definite stenosis of the aortic valve with calcium deposits. If any doubt arose as to the validity of the pathologic diagnosis the case was discarded. No measurement of valve circumference was set up as a limit, but in all instances it was less than normal. Precise quantitation of the degree of valvular obstruction was not feasible but from the description it was possible to classify the lesion as severe, moderate or minimal in every case. In many instances the qualifying discussion mentioned size of the opening in general terms. Stenosis was considered *severe* when the pathologist recorded it as such or the terms "only tip of finger is admitted by the aortic orifice" or "the aortic orifice is a mere slit" or "fish mouth". In a few cases only the tip of the small scissors used to open the coronary arteries could be passed through the existing aortic opening. *Moderate* stenosis included those cases when more than the tip of the finger could be inserted by the pathologist and includes those cases where "the aortic opening just admits one finger". By *mild* stenosis is meant those cases with definite narrowing of the aortic ring or with obstruction to the outflow of blood by calcified leaflets, the opening admitting one finger with ease despite gross evidence of stenosis. Immobility of valves gave further hints about the degree of stenosis.

After the entire series of cases had been selected from the files in the department of pathology, the hospital records were studied. In addition to the anatomical division based upon the degree of stenosis, the cases were divided into *Group I, the "cardiacs"* and *Group II, the "non-cardiacs"* on the basis of symptoms and course of the disease. If there was heart failure, or if the major com-

plaint was related directly to the heart, the case was put in Group I. While it was difficult to draw a sharp line in a few instances, this approach was used to throw light on the reason for the large number of cases where the diagnosis was missed. Since congestive failure did not occur in patients with subacute bacterial endocarditis they were put in Group II. All patients had heart disease. The possibilities for handling the data are many; and our choice has followed traditional lines for the most part. Some important aspects of aortic stenosis have been omitted or given scant consideration because of inadequate data.

Unlike many diseases with a better known clinical history, aortic stenosis as it is seen on the wards often has its sources shrouded in the mystery of dim memories and faint recollections. How and when it began are usually uncertain. The whole of its natural history cannot be delineated clearly from our data because the emphasis of observation is placed on the last chapter of the story, or the surprising discovery of stenosis at autopsy. Nonetheless, patients who have been observed at intervals supply some of the desired information and from their course we can get evidence bearing on prognosis. Not until careful health records are kept on large population groups throughout their lives will it be possible to fill some of the gaps in our knowledge.

The human substratum of our cases, influenced by the patient material of a municipal hospital, may not conform to that of the higher social and economic levels of private practice. Collections of autopsied cases by consulting cardiologists, however, rarely reach a large size. A complementary study of such cases would be very illuminating. Even 107 cases scarcely suffice for broad generalizations such as have been made from much smaller series. This limitation on our data should be borne in mind in evaluating our results, since we have not included a statistical manipulation of the figures themselves.

IV. BACKGROUND OF THE DISEASE

Age: The cases have been separated on the basis of age, sex, color, occupation and body build or type. Figure 1 gives the distribution by age, sex and color. The greatest frequency is found in the later decades of life but an occasional case appeared in the earlier decades. These facts are striking but their importance is emphasized by relating the age distribution of cases to that of the population of Cincinnati. Using the census figures for the years 1930 and 1940, which correspond well with the years covered by the study (1924-1946), the curves of Figures 2 and 3 show that aortic stenosis occurred with increasing frequency in age groups where the per cent of persons in the general population fell off sharply. If the risk of dying with aortic stenosis were the same at all ages the percentage of cases in each decade should correspond roughly with the percentage of population, that is, follow the line of unity. Figure 2 shows that in males aortic stenosis was relatively rare until the fifth decade when its incidence became nearly proportional to that of the population, with an increased frequency becoming progressively more pronounced in the sixth, seventh and eighth decades. After the age of 70 it was ten times as frequent as the proportion of the population in that age group might have indicated. Figure 3

reveals the same trend for females. Thus it is demonstrated that aortic stenosis as found in autopsy records is predominantly a disease of the older age groups. Assuming that the lesion confers no boon of longevity it may be concluded that it develops readily during the later periods of life; and though the pathologic background may commence at an early age the disease itself is evidently no

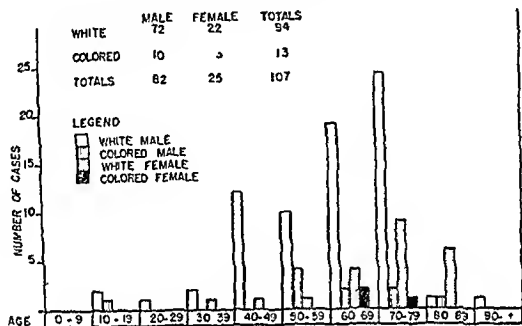


FIG. 1. DISTRIBUTION BY AGE, SEX, AND COLOR

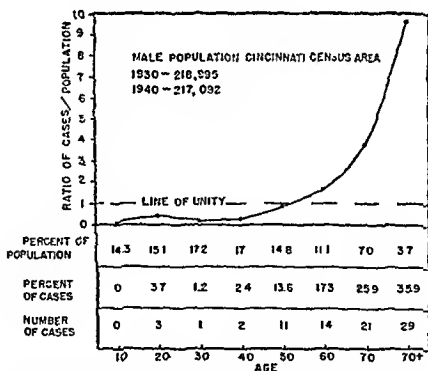


FIG. 2. DISTRIBUTION OF CASES BY AGE GROUPS: MALES

The figures for age represent decades, 10 referring to the first decade, 20 to the second and so on.

barrier to reaching an old age. Presumably it does shorten life in many who suffer from it.

Sex: Although males constituted only 48.1 per cent of the city population, 76.4 per cent of the cases were males with the corresponding figures for females 51.9 per cent and 23.6 per cent. Men outnumbered women 3 to 1. This is far out of line with the situation in acute rheumatic fever and rheumatic heart

disease where the sexes are affected about equally (74). Beyond the suggestion that endocrine factors may have an influence nothing is known of the cause of this disproportion. It is similar to the ratio of men and women with myocardial infarcts (5). The degree of stenosis in men tended to be more advanced than in women, and more men suffered from heart failure. There was a strong trend for heart failure to be associated with severe or moderate stenosis, and not with mild stenosis. The exceptions, however, were numerous and it is of interest that slightly more than one-fifth of the cases with severe and moderate stenosis were in Group II.

Color: It is reported (74) that the incidence of rheumatic fever is lower in Negroes than in whites. In this study Negroes comprised 11.4 per cent of the

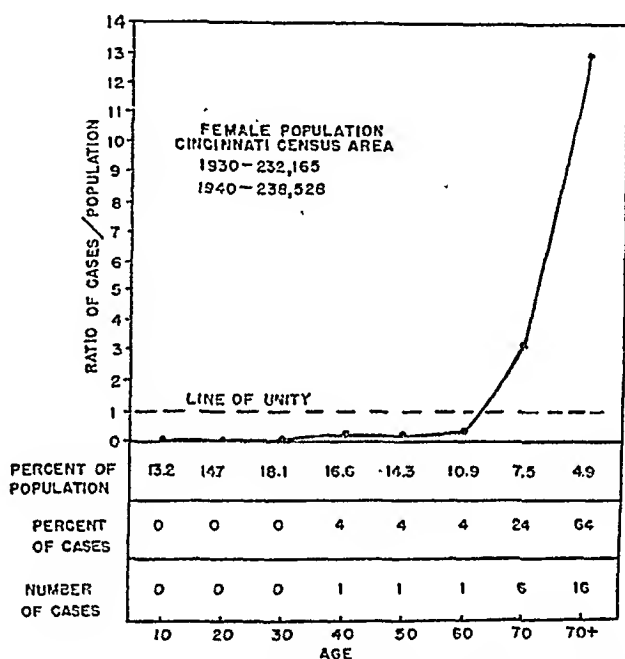


FIG. 3. DISTRIBUTION OF CASES BY AGE GROUPS: FEMALES

The figures for age represent decades, 10 referring to the first decade, 20 to the second and so on.

city population and 12.3 per cent of the cases giving no evidence of racial disposition or immunity to aortic stenosis. This cannot be cited as testimony for or against its rheumatic origin.

Occupation and anthropological type did not play any significant role in aortic stenosis. Many common occupations were represented. Type and body weight were distributed over the expected range, with normal or somewhat thin persons in slightly high frequency.

A comparison of color, sex and the severity of the lesion is made in Table 1 with separation of cardiac and non-cardiac cases. Forty-seven cases had advanced, 45 moderate and 15 mild stenosis. About $\frac{4}{5}$ of those with severe or moderate lesions fell into Group I while only $\frac{1}{3}$ of those with mild lesions fell into this group. Among white males 56 per cent had severe, 33 per cent moderate

and 11 per cent mild lesions. Grades of stenosis in white women were: 27 per cent severe, 55 per cent moderate and 18 per cent mild. In colored males 10 per cent had severe, 60 per cent moderate and 30 per cent mild lesions. The three colored females all had moderate stenosis. Seventy-eight per cent of the white males were in Group I as were 68 per cent of the white females, 40 per cent of the colored males and all 3 colored females.

Family History: A family history of aortic stenosis was not obtained and only one patient knew of another member of the family having rheumatic heart disease. A family history of hypertension, coronary artery disease or hemiplegia was obtained in 35 per cent of the cardiacs with severe stenosis but in only 10 per cent of those in other classes. This may be a clue to some underlying dis-

TABLE 1
Comparison of severity, color and sex in cardiac and non-cardiac cases

DEGREE OF STENOSIS	SEVERE		MODERATE		MILD	
	Group I	Group II	Group I	Group II	Group I	Group II
White Male	31	9	21	3	4	4
White Female	5	1	9	3	1	3
Colored Male	1	0	3	3	0	3
Colored Female	0	0	3	0	0	0
Total	37	10	36	9	5	10
White Male	40		24		8	
White Female	6		12		4	
Colored Male	1		6		3	
Colored Female	0		3		0	
Total	47		45		15	
	GROUP I—CARDIAC		GROUP II—NON-CARDIAC		TOTAL	
White Male	56		16		72	
White Female	15		7		22	
Colored Male	4		6		10	
Colored Female	3		0		3	
Total	78		29		107	

position to sclerosing vascular disease which eventuates in calcification of artery or valve. Since cases with complicating mitral stenosis were excluded the role of rheumatic fever cannot be evaluated in an unbiased manner. Even with this limitation a definite history of acute rheumatic fever was obtained in two-thirds of those with relevant data. Also, in view of the common discovery of mitral stenosis in this hospital with no history of recognized rheumatic fever our figures give formidable testimony favoring this disease as a precursor of aortic stenosis. That other etiological factors exist is possible.

Past History: Much controversy exists regarding the pathogenesis of aortic stenosis, particularly the calcific type. For that reason our information on the history of acute rheumatic fever in Table 2 is of interest. Only cases with a clear statement about presence or absence of attacks of acute rheumatic fever

were included. In 66 per cent there was a positive history. This evidence deserves even more emphasis in view of our exclusion from the study of cases with significant mitral stenosis. It does not prove that rheumatic heart disease is the necessary or invariable antecedent of calcific aortic stenosis.

The general medical history is recorded in Table 3 with separation into Group I and II and according to the severity of the lesion. Evidence of cardiac failure occurs in the Group I cases, with dyspnea in all, orthopnea in most and peripheral edema in many. Such signs and symptoms did occur in some of Group II, but

TABLE 2
History of acute rheumatic fever

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL	
	Severe	Moderate	Mild	Severe	Moderate	Mild		
Definite Positive History.....	14	14	1	2	1	1	33	66%
Definite Negative History.....	9	2	1	3	1	1	17	34%

TABLE 3
History

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Number of Cases.....	37	36	5	10	9	10	107
Dyspnea.....	37	31	4	1	4	2	79
Orthopnea.....	26	25	4	0	1	0	56
Edema.....	23	20	3	0	1	2	49
Cardiac Pain.....	17	20	2	1	0	0	40
Cough.....	21	15	2	0	0	0	38
Weakness.....	17	12	2	0	1	0	32
Dizziness.....	10	12	3	0	1	0	26
Abnormal Mental States.....	13	7	1	0	0	0	21
Nocturia.....	7	6	0	0	3	2	18
Syncope.....	5	8	2	0	1	1	17
Weight Loss.....	9	6	0	0	0	0	15
Palpitation.....	8	4	2	1	0	0	15
Epistaxis.....	5	1	0	2	1	0	9
Headache.....	5	2	0	0	0	0	7
Focal Cerebral Signs.....	1	1	2	0	0	0	4
Sweating.....	1	1	1	0	0	0	3

they had been mild, or did not figure in the condition for which the patient was hospitalized. Cough was encountered frequently and was associated with other signs of congestive failure rather than with infection. Cardiac pain occurred in half of the cardiac patients, and in many instances differed from the syndrome of classic anginal pain. Abnormal mental states were notable for their variety and changeability ranging from trifling petit mal like attacks to delirium or coma. Dizziness was more common than in other types of heart disease, and syncope was frequent. Unexplained weight loss was found in a number; and in many instances it was associated with weakness, which was common. Other symptoms

noted in the history occurred relatively infrequently. There were several instances of nosebleeds. The sweating and focal central nervous system signs were generally accompaniments of forward failure. In summary, the history of those who were admitted with manifest cardiac troubles gave evidence of both backward and forward failure. There was a tendency for congestive failure once established to be chronic, sometimes with gradual remissions, whereas the symptoms we have ascribed to forward failure were usually intermittent, often of only brief duration, though there was a trend toward increasing frequency as the disease progressed and the patient grew older.

Our division of cases into cardiac and non-cardiac classes implies that although failure of the heart is important in the natural history of the disease, aortic stenosis may not cripple the heart, and actually may be latent symptomatically. The history of the illness for which the patient was admitted reveals that dyspnea, orthopnea and dependent edema predominated in Group I and did not cause significant inconvenience in Group II. Similarly cough and cardiac pain affected Group I. There was a large element of dizziness, vertigo, syncope and mental symptoms in Group I patients. The presumptive evidence attributes such phenomena to a combination of inadequate cardiac output and vascular disease of the brain. Weight loss was found often, and was not accounted for by the usual causes. Bouts of sweating were mentioned often. Severe epistaxis was the precipitating cause of cardiac failure in 3 instances. While hemorrhage has been recognized as a rare factor in precipitating congestive failure or myocardial infarction (5) we have not seen it described as an agent provoking trouble in patients with aortic stenosis.

In Table 4 are listed some of the common findings in the history unrelated to the underlying disease. Alcohol addiction leads the list. We have no ready explanation of its frequency in this series of cases. The other diseases are not out of line with their incidence in adult admissions to this hospital.

V. COURSE PRIOR TO LAST HOSPITAL ADMISSION

The clinical course of each patient before the last hospital admission varied. Often it was like that of patients with other chronic valvular disease. Several patterns of symptoms emerged. There was no particular pattern of cardiac failure related to the extent of aortic stenosis. Table 5 lists some of the prominent features. Symptoms other than mild were not recorded for the 29 Group II patients and 19 did not have any cardiac symptoms. Two patients were subject to fits of dizziness and syncope. They accidentally fell and ultimately died of subdural hematomata.

Cardiac failure great enough to limit physical activity and present for periods varying from one month to several years occurred in 34 patients; 18 had severe aortic stenosis, 15 moderate and 1 mild. Repeated bouts of left and right sided heart failure were present in 10 patients. Eight of these had moderate aortic stenosis. Nineteen cardiac patients gave histories of the abrupt onset of cardiac failure 24 hours to a few days before admission. These patients all had many complaints due to mild cardiac insufficiency but not enough to interfere

with their usual work. Eleven of these 19 patients had severe aortic stenosis, 6 moderate and 2 mild. Histories of symptoms associated with cardiac insufficiency of recent origin (3 months or less) were obtained in 19 cardiac patients; 10 had severe aortic stenosis, 8 moderate and 1 mild.

Five patients were admitted with histories predominately of impaired mental function (confused, paranoid, delirious) for periods of a few days to one month. All had previous symptoms of mild cardiac failure. Three of them had severe aortic stenosis.

TABLE 4
History (miscellaneous)

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Severe Alcoholism.....	9	8	2	1	3	1	24
Gonorrhea.....	8	3	0	0	1	1	13
Treated and Untreated Syphilis.....	5	3	0	0	1	1	10
Frequent Colds.....	4	3	0	0	0	2	9
Hemoptysis.....	5	1	1	0	0	0	7
Pneumonia.....	0	0	0	1	2	2	5
Night Sweats.....	2	2	0	0	0	0	4
Asthma.....	2	2	0	0	0	0	4
Diabetes.....	2	1	0	0	0	0	3

TABLE 5
Clinical course prior to final hospital admission

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Chronic Congestive Failure.....	18	15	1	0	0	0	34
Rapid, Progressive, Unremitting Congestive Failure.....	11	6	2	0	0	0	19
Symptoms Related to Main Cause of Death—Endocarditis, Carcinoma, Peptic Ulcer, etc.....	0	0	0	9	4	6	19
Repeated Bouts of Congestive Failure.....	1	8	1	0	0	0	10
Mild Cardiac Symptoms.....	0	0	0	1	4	3	8
Severe Dizziness and Syncope.....	1	3	0	0	1*	1*	6
Mental Symptoms.....	3	1	1	0	0	0	5
Severe Epistaxis Precipitating Signs of Heart Failure.....	2	1	0	0	0	0	3
Cardiac Pain and Cardiac Asthma.....	0	2	0	0	0	0	2
Severe Cardiac Pain.....	1	0	0	0	0	0	1

* Falls resulting from syncope caused subdura hematoma.

Epistaxis of sufficient degree to precipitate cardiac failure occurred in 2 patients with severe aortic stenosis and 1 with moderate stenosis. Another patient who had cardiac pain for 7 years was an invalid for 2 years and 8 months because of the intractable pain provoked by effort. At autopsy no evidence of recent or old myocardial infarction was present but severe aortic stenosis was found. "Cardiac asthma" associated with cardiac pain necessitated hospital admission for 2 patients with moderate aortic stenosis. These symptoms and signs were present for 1 year in 1 patient and 1 month in the other.

Severe dizziness with repeated falls was the chief complaint of 4 patients who exhibited mild signs of cardiac failure. One of these patients had severe stenosis and gave a history of bad dizzy spells for 3 years. The remaining 3 patients had moderate aortic stenosis with dizziness present for periods of 2, 4 and 15 months.

Thus the clinical course was not unlike that of patients with other chronic valvular diseases but there was not exact correlation of severity or duration of congestive failure with degree of stenosis found at autopsy. A balance between the heart's capacity for work and the degree of valve obstruction must have determined the progress of the symptoms and signs, but this was not reflected in heart muscle hypertrophy in terms of weight. Increased average weight of the heart was associated with heart failure as well as with the valvular obstruction though there were some individual exceptions (Table 19). There were 34 patients who had experienced chronic congestive failure with limitation of activity but not total invalidism for periods ranging from 12 years to one month before their final admission. In 19 patients the signs and symptoms of heart failure were severe, commenced abruptly and lasted only a few weeks prior to admission. There were 10 patients who had numerous bouts of failure, often but not always requiring hospitalization. When compensation returned the adjustment was good and they were able to go about their accustomed activities with scant restriction.

There was a group of patients with acute or chronic cerebral disorders. There were 6 with severe dizziness or syncope which incapacitated them and 5 with mental disorders which varied in intensity and kind. Although the evidence is not direct these disorders probably are the result of inadequate cerebral circulation resulting from the aortic stenosis combined with vascular disease of the brain, the locale of which influenced the symptoms. There was no case in which carotid sinus sensitivity was found to induce syncope, and it was looked for a number of times.

There were 3 patients whose congestive failure had its clinical inception or took a decided turn for the worse following a copious nosebleed. Three others had a course characterized by cardiac pain which stringently limited their activity even though exercise did not regularly produce attacks. In 2 of these there was also severe cardiac asthma.

The majority of those in Group II had no symptoms of congestive heart failure although 8 of the 29 had mild dyspnea and edema. Nineteen were admitted for some intercurrent disease which was responsible for their death. In two cases death resulted from subdural hematoma following a fall in a syncopal attack.

VI. ADMISSION RATE

The average number of hospital admissions per patient for the entire series of cases was 1.4. There was a tendency for those with mild lesions to have more admissions (1.87) than those with moderate (1.36) or those with severe lesions (1.34). The reason for this is found in the large number of patients with

advanced stenosis whose only admission was the final one. The cardiac patients had a total of 117 admissions or 1.5 per person, the non-cardiacs had 35 admissions or 1.2 per person. A number of patients in each category of severity had several admissions, the largest number being 8.

Because season of admission in acute congestive failure and of onset of acute myocardial infarction show definite trends (7), the month of admission was examined. An evaluation of the data is difficult because of the relatively small number of admissions. The seasonal variation is presented in Figure 4. There was no special trend in the non-cardiacs but the cardiacs had a definite rise in admission rates in the months of September, October and November. This suggests that environmental stress may have influence in determining the onset of congestive failure in persons with aortic stenosis. The peak of admissions

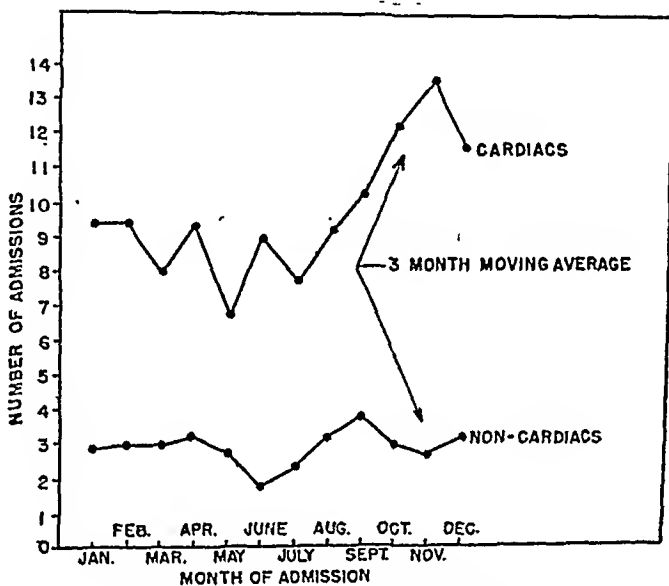


FIG. 4. MONTH OF ADMISSIONS

Number of admissions by months in Group I (cardiac) and Group II (non-cardiac) patients.

is two months earlier than the January peak of attacks of acute myocardial infarction (5, 7).

VII. STUDY OF THE PATIENT

Because the patient with aortic stenosis has a chronic disease which does not run a regular and predictable course, and which may be beset with a multitude of related and unrelated complications, the clinical picture is one of much variability. This is true from one patient to the next and is true of the same patient at different stages of his disease. Patients admitted to the wards of a municipal hospital are definitely and obviously sick as a general rule but those with a chronic valvular disorder may come in because of it or because of some intercurrent disease. Division of our cases into cardiac and non-cardiac groups revealed some errors in diagnostic awareness and some examples of oversight

in evaluating clinical manifestations of the disease. In more instances than we suspected, however, where the diagnosis was missed it was because the patient did not display the classical signs and symptoms. It was not rare for the heart to be strangely silent insofar as the so-called typical murmurs and thrills are concerned. While the subjective element in physical examination is an unmeasured variable we are convinced that the cases we are discussing had reasonably careful study, sometimes by visiting staff physicians, and usually by students and

TABLE 6
Chief complaints or disease on final hospital admission

DEGREE OF STENOSIS.....	SEVERE	MODERATE	MILD	TOTAL
Group I				
1. Dyspnea and Orthopnea.....	24	22	4	50
2. Cardiac Pain.....	4	5	0	9
3. Mental Disorders.....	4	3	0	7
4. Dizziness.....	2	3	0	5
5. Syncope.....	1	2	1	4
6. Epistaxis.....	2	1	0	3
Total.....	37	36	5	78
Group II				
1. Subacute Bacterial Endocarditis:				
Chills and Fever.....	4	2	1	
Cerebral Embolism.....	0	1	0	8
2. Injury:				
Subdural Hematoma.....	0	1	1	
Fracture.....	0	0	2	4
3. Abdominal Carcinoma.....	1	2	0	3
4. Hematemesis and Melena.....	2	1	0	3
5. Carcinoma of Breast.....	1	0	1	2
6. Meningitis.....	1	0	0	1
7. Carcinoma of Prostate.....	1	0	0	1
8. Hemiplegia.....	0	1	0	1
9. Mercury Poison.....	0	1	0	1
10. Urinary Tract Infection.....	0	0	1	1
11. Tuberculosis.....	0	0	1	1
12. Carcinoma of Cervix.....	0	0	1	1
13. Lobar Pneumonia.....	0	0	1	1
14. Fracture of Hip.....	0	0	1	1
Total.....	10	9	10	29

house officers. For varying periods during the past 10 years one or both of us have seen a number of the patients.

Cause of Admission: It is customary in this hospital to place on each patient's record the major complaint or cause of admission, often in his own words. In Table 6 we have listed the leading cause of entry into the hospital according to Group I or II status and the severity of the aortic stenosis. This table gives one of the main reasons for separation into cardiac and non-cardiac groups.

Group I: Dyspnea and orthopnea were the chief complaint in 65 per cent of the cases. Thus congestive failure was the most prominent cause of admission

to the hospital. Cardiac pain, the next most common complaint, accounted for only 12 per cent. Although the term *angina pectoris* often was used to describe this condition it was usually of a bizarre type and differed from classic *angina*. Mental disturbance of wide variety was the presenting disorder in 9 per cent of the cases. It was usually sufficiently severe to cause admission to the psychiatric wards. No standard pattern existed; coma, delirium, confusion, disorientation and mixtures of such conditions were the usual findings. Dizziness and vertigo accounted for 6 per cent of the cardiac admissions. They were complained of often even when they were not the major presenting symptoms. Syncopal attacks constituted the chief disturbance in 5 per cent. They were sometimes severe, lasting for many minutes, and their repetition was so disabling as to cause the victim to seek relief in the hospital. One patient was admitted for repeated attacks of cardiac asthma and 3 had a severe nosebleed.

Group II: Persons who were admitted for non-cardiac complaints fell into many groups—one with various forms of injury, another with manifestations of subacute bacterial endocarditis and the remainder a nondescript miscellany. Subacute bacterial endocarditis occurred with signs of an infection or had its clinical debut with a cerebral embolus. Another group contained victims of an injury unwittingly self-inflicted because of a fall induced by syncope. Cranial injury with subdural hematoma or fractures of leg or arm occurred, a situation which has not been emphasized heretofore. The remainder consists of such diseases as are common in older people. Aortic stenosis had been asymptomatic or productive of only trivial complaints which were overshadowed by some other more obvious disease. The clientele of a municipal hospital may perceive, evaluate and describe symptoms inadequately. The case records, while some are faulty, are unbiased. Preconceived notions of what should constitute the symptomatology of the disease cannot have influenced the data when the diagnosis was missed. A summary of the most common signs and symptoms is given in Figure 5.

In Group I it was noted that the onset of congestive failure frequently followed directly or within hours some unaccustomed activity or vigorous exercise. Running, lifting and more protracted work were encountered relatively frequently and in persons with several admissions for congestive failure such a history was common. This was found also in persons with syncope which usually followed exercise, or interrupted it, but nothing was found to explain why syncope was thus provoked only occasionally.

Discussion: In Table 6 the cause of admission has been given in detail. Those with cardiac symptoms were admitted for much the same conditions which had characterized their course prior to hospitalization. Congestive failure accounted for 68 per cent, signs ascribed to forward failure 20 per cent and cardiac pain accounted for the remaining 12 per cent. Untoward exercise or strain preceded a worsening of the signs of failure in many cases. Subacute bacterial endocarditis, carcinoma and injury caused most admissions among those who had not been troubled with congestive failure and the rest of the non-cardiac group was a motley collection of disorders, most of them the degenerative diseases of older years.

VIII. PHYSICAL EXAMINATION

There was no single invariable sign on physical examination although the major manifestations of patients with aortic stenosis centered around the heart. Failure of either backward or forward type predominated. There was no characteristic temperature curve. Fever, when encountered, and especially when of appreciable magnitude, indicated some complication such as pneumonia, bacterial endocarditis or pulmonary infarction, and in a few instances, myocardial infarction.

Pulse, Respiration and Blood Pressure: There was no regular pattern of pulse rate. As a group the cardiacs had tachycardia and an average rate of 96 while the average for Group II was 86. The means and extremes of cases

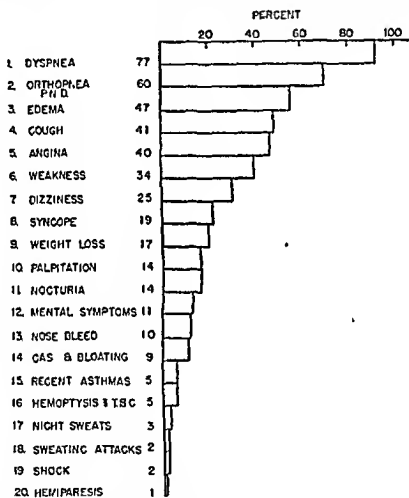


FIG. 5. SIGNS AND SYMPTOMS IN 84 CASES

The term angina refers to cardiac pain. Recent asthmas refers to attacks of cardiac asthma.

in various subdivisions are presented in Table 7. Those with a moderate degree of stenosis in Group II had a more rapid average heart rate than those with severe stenosis. There were several cases of bacterial endocarditis in this group. No extremely rapid pulse rates were recorded and marked tachycardia was not prevalent. There was no instance where syncope was associated with extremely slow pulse rate and the asystole of the Adams-Stokes attack was nowhere observed. As far as can be determined from the records, the pulse rate was accelerated in fair proportion to the extent of congestive failure.

Respiratory rate followed the general trend of tachypnea in the presence of congestive failure, and Table 7 reveals that just as dyspnea was a major complaint of the cardiacs, so also was rapid respiration the common finding.

Discussion: Tachypnea was associated with dyspnea in most cases. The straight line of low respiratory rates at 20 probably indicates a foible of the nurses' recording of respiration. Our personal observations give assurance that a rapid rate of breathing was usual, and was a fair index of the severity of congestive failure.

Blood pressure did not conform to the classic description for aortic stenosis (2, 17, 21, 28, 38, 54, 75, 78) and there was no standard. Reference to Figure 6 and Table 7 emphasizes two points—high diastolic pressures were common and high pulse pressures with low diastolic pressures were common. Severe systolic hypertension was not found but in every class one or more had systolic

TABLE 7
Pulse, respiration and blood pressure

Degree of Stenosis.....	GROUP I			GROUP II		
	Severe	Moderate	Mild	Severe	Moderate	Mild
Pulse Rate:						
Low.....	60	66	70	48	72	60
Average.....	94	97	98	86	95†	77
High.....	140	135	130	130	120	130
Respiratory Rate:						
Low.....	20	20	20	20	20	20
Average.....	32	32	32	24	29	25
High.....	55	44	46	38	36	36
Blood Pressure:						
Systolic:						
Low.....	60	60‡	138	98**	104	102
Average.....	120*	140	163	130	125	140
High.....	174	190	190	190	190	180
Diastolic:						
Low.....	50	30	80	40	35	38
Average.....	77*	73	95	69	69	67
High.....	110	110	112	110	94	90
Pulse Pressure:						
Low.....	12	28	26	30	10	42
Average.....	46	67	64	61	56	68
High.....	90	120	90	110	85	100

* No B.P. could be obtained in 2 patients.

† Increased by tachycardia in 4 cases of bacterial endocarditis.

‡ No data on 1 case.

** No data on 2 cases.

blood pressures in the range of 180 to 190 mm. Hg. Among Group I patients, those with severe stenosis had a lower average systolic pressure and those with mild stenosis had a higher average systolic pressure. To some extent this same variation existed in diastolic pressures. In several instances blood pressure readings so different from those described in typical aortic stenosis had been an influence against the correct diagnosis. There were a number of patients whose pulse was small, and of the slowly rising plateau type, and whose blood pressure showed the relatively low systolic and small pulse pressure. They were in the definite minority. Reliance on these signs was more a hindrance than a help in diagnosis because of their rareness. When present they lend confirmation but their absence is no reason for excluding the diagnosis.

Heart: Enlargement of the heart was noted by percussion or by palpation of a displaced apical impulse in 78 per cent of Group I and 52 per cent of Group II. There was no close correlation between heart size, as detected on the physical examination, and the degree of stenosis (Table 8). This is in sharp contrast to the close relationship found between heart weight and degree of stenosis at autopsy. While cardiac dilatation may account for part of this discrepancy it is unlikely that it was the only factor. Our study does not provide any convincing explanation.

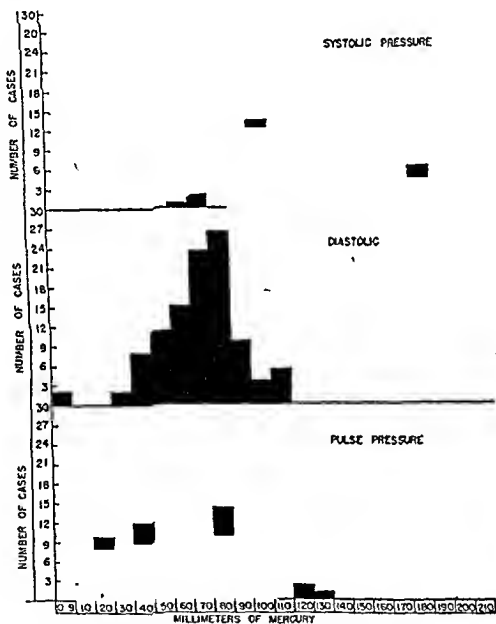


FIG. 6. DISTRIBUTION OF BLOOD PRESSURES

The blood pressure figures were obtained from the first reading after admission.

Auscultation: In 16 instances respiratory noises were so loud that the heart tones were obscured. In those with satisfactory auscultation the first heart sound at the apex was muffled in the great majority of cases although in some of the cardiacs it was described as normal. Where comment was available in Group II patients the first sound at the apex was muffled but most often it was not described at all. As a general rule, therefore, the apical sounds were not clear and pure, but tended to be soft or muffled. At the base, the aortic second sound was absent a number of times, was soft or muffled in some but in a few was found to be booming in quality. Less information is available regarding the

sound of pulmonary valve closure. In four cases it was not heard, in 9 it was muffled and in 9 it was normal. A comparison of aortic and pulmonic second sounds reveals that P_2 exceeded A_2 in 17 cases whereas A_2 exceeded P_2 in 16 and in 7 they were of equal intensity. The majority of the cases where P_2 was loudest occurred in the cardiacs with severe stenosis. Abnormality of the aortic second sound, especially at the base, was helpful in establishing the proper diagnosis, and this was particularly true where it was faint or absent. In some cases, where the sound at the right of the sternum in the second interspace was loud, the valve was rigid and it is not possible that it contributed a closure sound. In such cases the closure of the pulmonic valve must have provided the sound which was clearly and sometimes most loudly heard to the right of the sternum.

TABLE 8
Physical examination of the heart: size and sounds

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Enlarged.....	29	28	4	5	7	3	76
Apical Sounds:							
1st Sound Normal.....	7	4	0	0	0	0	11
1st Sound Muffled.....	30	10	4	1	4	1	50
No Comment.....	0	22	1	9	5	9	46
2nd Sound Normal.....	7	5	0	0	0	0	12
2nd Sound Muffled, Soft.....	30	8	0	0	0	0	38
No Comment.....	0	23	5	10	9	10	57
Basal Sounds:							
A_2 Absent.....	10	4	5	3	1	0	23
A_2 Muffled.....	7	7	0	0	0	0	14
A_2 Booming.....	2	4	0	0	0	0	6
A_2 Not Described.....	18	21	0	7	8	10	64
P_2 Normal.....	4	5	0	0	0	0	9
P_2 Muffled.....	7	2	0	0	0	0	9
P_2 Absent.....	2	1	0	1	0	0	4
P_2 Not Described.....	24	28	5	9	9	10	85
$A_2 > P_2$	4	6	2	1	2	1	16
$P_2 > A_2$	11	5	0	1	0	0	17
$A_2 = P_2$	4	2	1	0	0	0	7
Respiratory Noises Obscured Heart Sounds.....	8	8	0	0	0	0	16

Murmurs: The rough systolic murmur at the base, transmitted to the neck, and the systolic thrill have occupied the center of the clinical picture of aortic stenosis of the textbook. One of the complicating factors in most such cases described has been the frequent association of mitral valve disease with stenosis or regurgitation or both. The present series of cases, because of the exclusion of deformity of other valves, permits a more just evaluation of the murmurs and thrill produced by stenosis, with or without regurgitation of the aortic valve as the exclusive lesion.

Basal murmurs were frequent (See Table 9). The loud, harsh systolic murmur heard best over the aortic area was described 78 times and a soft murmur was heard 11 times. Transmission to the neck was noted specifically in 42 instances, down the sternum 3 times and to the left axilla once. No transmission

was recorded in 45 cases, and there was no transmission to the back. Diastolic murmurs were heard at the base 35 times, and were more often soft than loud. Eleven were transmitted to the apex and 3 to the xiphoid. The murmurs at the base were heard more frequently, were louder and were more widely trans-

TABLE 9
Murmurs

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Apical:							
Systolic:							
Harsh, Loud.....	31	23	2	7	5	3	71
Soft.....	3	6	2	2	2	2	17
Transmitted to Axilla.....	15	9	1	2	1	2	30
Transmitted to Base.....	2	5	0	0	2	0	9
No Transmission Recorded.....	17	15	3	7	4	3	49
Diastolic:							
Harsh, Loud.....	2	3	2	1	1	1	10
Soft.....	9	6	1	2	2	0	20
Transmitted to Back.....	5	1	0	0	1	0	7
Transmitted to Axilla.....	2	1	0	0	0	0	3
Transmitted to Neck.....	0	0	2	0	0	0	2
No Transmission.....	9	9	1	3	2	0	24
Basal:							
Systolic:							
Aortic—Harsh, Loud.....	30	20	2	6	7	3	78
Aortic—Soft.....	2	2	1	2	1	2	11
Transmitted to Neck.....	16	15	2	6	2	1	42
Transmitted to Xiphoid.....	2	0	0	0	1	0	3
Transmitted to Axilla.....	1	0	0	0	0	0	1
No Transmission.....	15	17	1	2	5	5	45
Diastolic:							
Aortic—Harsh, Loud.....	4	6	0	2	2	1	15
Aortic—Soft.....	13	6	0	1	0	0	20
Transmitted to Apex.....	5	4	0	1	0	1	11
.....	1	1	0	1	0	0	3
.....	11	7	0	1	0	0	19
.....	3	0	0	1	0	0	4
At the Apex:							
Systolic.....	1	1	1	0	0	0	3
Diastolic.....	4	1	0	1	0	0	6
Thrills, Basal.....	8	7	0	3	2	1	21
Thrills, Tr. to Neck.....	6	5	0	0	0	0	11
Thrills, Apical and Basal.....	4	5	0	2	0	0	11
Thrills, Apical Only.....	1	1	0	0	0	0	2
Extrasystoles.....	7	11	1	2	0	1	22
Auricular Fibrillation Clinical.....	3	4	0	0	0	0	7
Gallop Rhythm.....	1	1	2	0	0	1	5
Respiratory Noise Obscured.....	3	2	0	0	0	0	5

mitted when the degree of stenosis was severe or moderate than when it was mild.

Apical systolic murmurs were heard in 88 cases or 82 per cent. Four-fifths of the murmurs were harsh and loud while the remainder were soft. Murmurs were heard more frequently with severe grades of stenosis, and were also louder. Transmission to the axilla was noted in 30 per cent. What was described as

transmission to the base indicated that the murmur was louder at apex than base and could be heard as the stethoscope was moved diagonally from apex up to the base. Transmission was not noted in more than half. A diastolic murmur at the apex was heard in 30 patients but was loud in only 10. In 2 cases it was heard in the neck and in 3 in the axilla while in 7 it was transmitted to the back. This somewhat unusual transmission is presumably indicative of a large heart which approached the wall of the chest posteriorly. Transmission was absent twice as often as it was present.

In 4 instances the systolic murmur was heard best over the pulmonic area or was confined to it. Since the pulmonic valve was normal this murmur doubtless arose from the aortic valve deformity but because of the heart's location or rotation the murmur was heard in the region where pulmonic valve noises are commonly heard best. This is in contrast to the transmission of pulmonic valve closure sound sometimes heard in the area overlying the aortic valve when mechanical closure of the aortic valve was anatomically impossible.

So-called tricuspid systolic murmurs were heard 3 times and diastolic murmurs were heard 6 times. Since there was no evidence of tricuspid stenosis or regurgitation on examining the heart at autopsy, these sounds may have arisen from the aortic valve and had a peculiar exaggeration in the tricuspid valve area. It is possible that functional tricuspid regurgitation was present, though no pulsation was noted in the sometimes very large livers.

Thrills were palpated at the base alone 21 times, at the apex as well as base 11 times and in 2 cases were felt at the apex only. All were systolic in time. Just one-half of the thrills felt at the base were transmitted to the neck. There was good agreement between degree of stenosis and incidence, intensity and transmission of thrills.

Rhythm: Extrasystoles were noted on auscultation in 22 cases and a grossly irregular rhythm was found in 7. Gallop rhythm was observed in only 5 cases.

Discussion: Cardiac sounds and murmurs departed from the classical description of the disease in a number of cases. In 82 per cent of the cases the first sound at the apex was muffled. The second sound at the apex was muffled, soft or indistinct in 76 per cent. There is relatively little (21, 26, 68) comment in the literature on these sounds as noted at the apex, probably because the signs at the base have been given so much attention. At the base there was no sound of aortic valve closure in 53 per cent of the cases with adequately described sounds, in 33 per cent the sound was muffled while in the remaining 14 per cent the sound was described as booming. Since in the great majority of cases the aortic valve was fixed and incapable of closing it is logical to assume that the loud sound heard over the aortic valve was contributed by the closure of the pulmonic valve. This interpretation is strengthened by the observation that in many such cases the second sound was intensified in association with pulmonary congestion. Descriptions of the pulmonic second sound are inadequate for analysis but a comparison of the aortic and pulmonic second sounds revealed $P_2 > A_2$ in 43 per cent, equal in 17 per cent and $A_2 > P_2$ in 40 per cent. As a general rule abnormality of the first heart sound at the apex was common. Even more common and helpful in diagnosis was absence or impurity of the

aortic closure sound but in some cases a loud sound was heard at the base over the aortic area. One should not demand, therefore, absence or decreased intensity of the second heart sound over the aortic valve in establishing the diagnosis of aortic stenosis.

Systolic murmurs were heard at the cardiac apex in 82 per cent of the cases, and four-fifths of them were loud and harsh. In one-third the murmur was transmitted to the axilla. Ten per cent of those loudest at the apex were transmitted to the base. Diastolic murmurs were heard at the apex in 30 cases, and it is probable that most or all of these had their genesis in aortic regurgitation. Systolic murmurs were heard at the base in only 83 per cent of the cases. The murmur's intensity was usually related to the degree of stenosis. Transmission to the neck was noted in slightly more than half of those with a basal systolic murmur. Omitting those in whom the murmurs may have been obscured or obliterated by loud respiratory sounds 12 per cent had no basal systolic murmur. Absence of this murmur was rarer in cardiacs (8 per cent) than in non-cardiacs (24 per cent) and the murmur was absent in relatively more with mild than moderate or severe stenosis. Nevertheless, in some patients with severe stenosis, with or without congestive failure, no basal systolic murmur was heard. We cannot account for all of these instances but in a number the murmur was absent during a period when the heart was failing acutely.

Failure to recognize the significance of a basal systolic murmur, even with upward transmission, indicates the degree to which the systolic murmur had fallen in importance. This was probably a misconstruction of the change in interpretation of apical systolic murmurs (26). Since our interest in aortic stenosis here, there has been too great a swing of the pendulum in the other direction. Aortic stenosis is diagnosed too readily by the present house staff.

Diastolic murmurs were heard best at the base in a third of the cases and were not transmitted to the neck, although some were heard widely over the precordium. This murmur was like that of aortic regurgitation, and this diagnosis was made in 8 per cent of the cases. The presence of a diastolic murmur, low diastolic blood pressure, Corrigan pulse and capillary pulsation should not militate against the diagnosis of aortic stenosis.

The murmurs described as pulmonic or tricuspid in all probability came from the diseased aortic valve; possibly from a dilated mitral valve. In no case was a murmur heard only in these regions and not over the aortic valve.

Other Signs: Physical signs which occurred with frequency gave further testimony of congestive failure (See Table 10). Rales were heard in the lower portion of the lungs, especially at the back, in 73 cases, and this sign of failure was more common where the degree of stenosis was severe or moderate. Rales were heard in some patients in Group II. Dependent edema had much the same incidence, and was associated with congestive rales in the lungs. Enlargement of the liver was noted in slightly more than half of the cases. Cyanosis, another concomitant of congestive failure, was noted in half of the cases. As a general rule these indications of congestion all appeared in a given patient but there was some irregularity.

Sweating, as a prominent feature of the disorder on admission, was encountered

in 35 cases. It was usually associated with a shock like condition. Its nature is not clear: whether a cerebral reflex or whether it was another sign of acute forward failure and incipient or established medical shock. Pallor was noted only rarely, but when combined with cyanosis and sweating created an impressive clinical picture.

Hydrothorax was found on physical examination 21 times, in 12 on the right side only, in 7 on both sides and twice on the left side only. Gross increase in venous pressure as evidenced by distended neck veins occurred in 14 cases, all Group I cases with either severe or moderate stenosis. Ascites occurred 10 times, and only in those with chronic congestion of the liver. The spleen was felt in 4. Jaundice occurred twice. One patient had symptoms of a focal central nervous system disorder on admission which cleared up while he was in the hospital.

TABLE 10
Physical signs

	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Degree of Stenosis.....							
Basal Rales in Lungs.....	32	30 (1)	2 (1)	2	4 (1)	2	73 (3)
Dependent Edema.....	25	29	2	2	5	0	63
Enlarged Liver.....	23	22	4	2	4	1	56
Cyanosis.....	27 (8)	22 (9)	1 (3)	0 (4)	1 (1)	2	53 (25)
Sweating.....	17 (9)	12 (1)	1	3 (1)	2	0	35 (11)
Hydrothorax:							
Right Alone.....	6	4	1	0	1	0	12
Both Sides.....	2	4	0	0	1	0	7
Left Alone.....	2	0	0	0	0	0	2
Confusion, Delirium and Coma.....	9	6	1	2	2	1	21
Neck Veins Distended.....	8	6	0	0	0	0	14
Ascites.....	3	5	0	0	1	1	10
Palpable Spleen.....	0	1	0	2	1	0	4
Pallor.....	3	0	0	1	0	0	4
Jaundice.....	0	2	0	0	0	0	2
Focal CNS.....	0	0	1	0	0	0	1

Figures in parentheses indicate additional cases where the sign appeared in the terminal stages of the disease.

Discussion: Physical signs of congestive failure prevailed among the cardiacs but were not confined to those who were admitted with congestive failure. As a rule the signs were more frequent and more pronounced among those with considerable valvular obstruction. Basal rales were heard in the great majority of those with failure, and most patients with rales had dependent edema. Enlargement and tenderness of the liver were only slightly less prevalent. Cyanosis occurred with about the same frequency early during hospitalization but appeared during terminal stages of the disease in another fairly large group. Other signs of congestive failure include hydrothorax which was found 21 times. It was confined to the right side in 57 per cent of the cases, bilateral in 33 per cent and left sided only in the remaining 10 per cent. These figures should be compared with the findings at autopsy. Ascites was noted 10 times and the spleen was felt in 4 patients only one of whom had bacterial endocarditis.

Sweating, a sign which has been given little attention in discussions of this subject occurred in a third of the cases at the time of initial examination and appeared later in an additional 10 per cent. It was severe, literally drenching the patient. Often but not always it was associated with other signs of shock and forward failure. In others it occurred in association with bizarre symptoms referable to cerebral anoxia and here also forward failure seems to be the likeliest cause.

Cerebral disorders, other than acute episodes of syncope or dizziness, were important clinical features in 20 per cent of the patients. Coma, delirium and stupor, as well as focal neurological signs sometimes completely dominated the symptom complex with which the patient came to the hospital. In a high proportion of such cases the patient was admitted to or transferred to the psychiatric wards for treatment, without recognition of the nature of the underlying disease. While it is well known that clouding of the sensorium and focal neurological signs may characterize such acute cardiac disorders as myocardial infarction (5, 6), the cardiac lesion is more likely to be recognized as a contributing factor. It appears that in patients with aortic stenosis we have another group with primarily cardiac disease in which symptoms are produced at a distance when cardiac output is embarrassed. Here, too, vascular disease in the brain provides the basis for symptoms and signs by its pattern of focal, irregular or generalized cerebral vascular sclerosis. While it is probable that the basis for syncopal attacks is similar, a detailed study of the disordered vascular architecture of the brain has not been made to demonstrate it. Nor do our data throw any light on the possibility that particular distributions of lesions in the arteries of the brain may give a morphologic explanation for sudden death.

IX. X-RAY FINDINGS

The x-ray studies are reported in Table 11. The heart was found to be enlarged in 75 per cent of the patients studied by x-ray and there was fair agreement with the estimate of size by physical examination. Hydrothorax was detected 12 times and the distribution as to side involved bore out the physical examination. Pulmonary congestion was found much less often than the clinical signs of rales at the lung bases. Where the signs on auscultation were pronounced, x-ray evidence usually was found in addition. Enlargement of the aorta, which would not be expected in aortic stenosis on a priori grounds, was found in x-ray or fluoroscopic examination 8 times. In such instances there was systolic hypertension or aortic regurgitation, or both. It was found only with moderate or severe stenosis, not with mild degrees.

Calcification of the aortic valves seen fluoroscopically and sometimes again verified by x-ray films (23, 81) is a valuable confirmation of the diagnosis of aortic stenosis. It was found in 5 out of 11 cases studied carefully under the fluoroscope but was not seen on the films.

Discussion: X-ray studies made on our patients were not pursued in routine fashion, and were necessarily limited because of the severity of the illness. The heart was enlarged according to the criteria used in 75 per cent of the 44 patients with a satisfactory examination. There was excellent agreement between the

information gained by physical examination and x-ray study. Cardiac enlargement was positively related to the degree of stenosis and to the presence of heart failure. Calcification of the aortic valves was demonstrated only 5 out of 11 times, but systematic study was not undertaken. Gross enlargement of the aorta was found in 18 per cent of those x-rayed. This was generally found in association with aortic regurgitation or systolic hypertension or both.

TABLE 11
X-ray and fluoroscopic findings

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Heart Enlarged.....	15	9	0	2	5	2	33
Heart Not Enlarged.....	1	3	1	3	0	3	11
Hydrothorax:							
Right.....	3	2	0	0	1	0	6
Both.....	2	2	0	0	1	0	5
Left.....	1	0	0	0	0	0	1
Pulmonary Congestion.....	1	6	0	1	0	0	8
Enlarged Aorta.....	3	2	0	0	3	0	8
Calcified Aortic Valves (Fluoroscopic).....	4	0	0	1	0	0	5
Pulmonary Infarct.....	1	1	0	0	0	0	2

TABLE 12
Laboratory data

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Albuminuria.....	32	30	4	6	7	6	85
No Albuminuria.....	5	6	1	4	2	4	22
RBC Normal.....	29	24	5	7	6	8	79
RBC Above 6M.....	2	0	0	0	0	0	2
RBC Below 4M.....	6	12	0	3	3	2	26
Elevated WBC.....	19	17	3	6	3	6	54
Elevated BUN.....	17 (4)	14 (5)	2	3	4	4	44 (9)
Positive Kahn.....	4	4	1	0	1	5	15
High Venous Pressure.....	3	1	0	0	0	0	4

Parentheses indicate number of cases where the finding occurred terminally.

X. LABORATORY STUDIES

The ordinary tests from the laboratory were of little help in establishing a diagnosis (Table 12). Many abnormal findings reflected some complication or some intercurrent condition. Albuminuria was noted in 79 per cent of the patients on admission and was more common in the cardiacs. In most instances it was slight or moderate. Many times it was associated with congestive failure but sometimes it was found where the prominent disability was forward failure. Blood urea nitrogen was found to be elevated in 29 of 36 cardiacs and 10 of 12 non-cardiacs. Circumstances complicating the course of the disease make it

hard to ascribe this to any isolated cause. Renal congestion, pyelonephritis, forward failure, hypertension, arterio-nephrosclerosis, renal infarcts and acute myocardial infarction or some combination usually were severe enough to account for nitrogen retention. Red cell counts were generally in the range of normal. In 26 cases there was a tendency to anemia, but except in those with bacterial endocarditis it was not severe. In several it followed one or more episodes of blood loss. Elevation of the leucocyte count was found 45 times. It was associated with some form of infection in most cases, and in no instance could it be ascribed to congestive failure alone. Just as did fever, leucocytosis occurred in bacterial endocarditis and myocardial infarction. Sometimes the puzzling nature of fever and leucocytosis was removed when pulmonary infarcts were discovered at autopsy.

Syphilis, as indicated by a positive Kahn or Wasserman reaction in the blood, occurred in 15 patients, 14 per cent. While we have no figures on the incidence of syphilis throughout the whole period, tabulation from the obstetrical service for the years 1939 through 1946 gives an incidence of 12 per cent positive reactions. Of approximately 11,000 determinations made in the hospital laboratory in 1944, 13.98 per cent were positive. Thus the incidence of syphilis in our patients with aortic stenosis is in keeping with the hospital experience. Actually the percentage may be somewhat high in our cases since there were relatively more white patients than Negroes and the percentage of positive serological reactions in Negroes is 2-3 times higher than in whites in this hospital. Syphilitic disease of the aortic valve was not found.

XI. ELECTROCARDIOGRAMS

There is no electrocardiographic sign pathognomonic of aortic stenosis. Such changes as occur may be ascribed to the underlying disease, the dynamic alterations which result from the burden thrown on the left ventricle (4), myocardial ischemia and associated changes such as coronary arteriosclerosis. The findings in 111 electrocardiograms taken in 53 cases are found in Table 13. Rhythm was regular in 41 cases, extrasystoles occurred twice and auricular fibrillation existed in 10. This incidence of fibrillation in 19 per cent of the cases is much higher than has been reported in other series (21, 28). Left axis deviation occurred in 74 per cent of the cases, and in 8 cases a diagnosis of left ventricular strain was made (4). Left bundle branch block occurred 10 times, complete heart block and shifting pacemaker once each. There was prolongation of the P-R interval beyond 0.2 seconds in 16 cases. Inversion of T waves in various leads and combinations of leads was common. Signs of myocardial damage, conduction defects and arrhythmia were much more common in cardiac than non-cardiac cases, and were more frequent with severe than with mild or moderate stenosis.

XII. HOSPITAL COURSE

Certain features of the hospital course of the 107 patients have been listed in Table 14. Respiratory distress of some kind occurred in every one of the Group I patients and was severe in 60 of 78, while severe respiratory distress was noted

in only 4 of the 29 in Group II. In these it was related to pulmonary disease. Once congestive failure became established, respiratory distress was severe regardless of the degree of stenosis.

TABLE 13
Electrocardiograms

	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Degree of Stenosis.....							
Rhythm:							
Regular.....	18*	13	1	4	5	0	41
Extrasystoles.....	0	1	1	0	0	0	2
Auricular Fibrillation.....	4	4	2	0	0	0	10
Left Axis Deviation.....	17	12	3	3	4	0	39
No Axis Deviation.....	5	6	1	1	1	0	14
L.B.B.B.....	3	3	3	0	1	0	10
L.V.P.....	6	2	0	0	0	0	8
Inversion of T ₁ or T ₁ and T ₂	12	9	3	1	1	0	26
Inversion of T ₁ , T ₂ and T ₃	4	3	0	2	1	0	10
Inversion of T ₂ and T ₃	1	2	0	0	1	0	4
Complete Heart Block.....	0	0	0	1	0	0	1
Prolonged P.R. 0.20-0.26 sec.....	0	5	1	2	2	0	16
Shifting Pacemaker.....	1	0	0	0	0	0	1
Number of Cases.....	22	18	4	4	5	0	53
Number of E.K.G.'s.....	43	37	12	11	8	0	111

* Numbers indicate cases rather than individual electrocardiograms.

TABLE 14
Course

	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Degree of Stenosis.....							
Severe Respiratory Distress.....	31	24	5	2	1	1	64
Bouts of Orthopnea, Flushing, Sweating, Cyanosis, Restlessness, Confusion.....	16	10	1	1	0	0	28
Terminal Coma.....	8	6	1	3	3	2	23
Sudden Death.....	10	11	1	0	0	0	22
Rapid Downhill Course Without Response to Rx.....	8	8	1	0	0	0	17
Cardiac Pain on Ward.....	5	4	0	1	0	0	10
Sudden Downhill Course.....	3	5	1	0	0	0	9
Confusion, Stupor, Delirium, Hallucination.....	4	3	0	1	0	0	8
Pneumonia.....	3	0	0	2	0	1	6
Convulsions.....	4	0	1	0	0	0	5
Continuous Shock State Without Response.....	2	0	0	0	0	0	2
Extreme Dizziness.....	0	0	1	0	0	0	1
Syncope.....	1	0	0	0	0	0	1
Hemiplegia.....	1	0	0	0	0	0	1

In 28 patients there were very striking episodes of flushing, sweating, cyanosis, restlessness and confusion. There was usually some dyspnea and orthopnea. These spells would come on suddenly without any clear-cut provoking stimulus. They would last anywhere from minutes to hours. They did not respond well

to oxygen therapy. In many persons so affected there was gradual recovery but in some the attack vanished as quickly as it had come without regard to the kind or degree of treatment. The sweating was especially pronounced, literally drenching the gown and bed clothes. Occasionally there was pallor rather than flushing. Some degree of shock was present at times, and the blood pressure was usually low during the attack. All but one of these patients were in Group I; 17 had severe, 10 moderate and one mild stenosis. It is surmised that this phenomenon was related to forward failure and inadequate cardiac output. Two additional patients were admitted in shock, and died without responding to treatment.

Although cardiac pain had been experienced by 40 patients, only 9 of these had further bouts of pain after admission to the hospital. There was one additional patient in the non-cardiac class who experienced pain while in bed.

Symptoms of mental aberration dominated the course of 8 patients. Confusion, stupor, delirium, hallucinations and disorientation occurred variously combined. Five other patients had convulsions. All but one of these 13 patients had severe or moderate stenosis. Severe dizziness was noted in one cardiac patient with only mild stenosis; one with severe stenosis had syncope without dizziness and another developed hemiplegia without an acute vascular lesion of the brain to explain it. The evidence suggests that such episodes or states result from cerebral anoxia ensuing upon inadequate cardiac output through the obstructed valve. Their diffuse or focal expression is best explained on the basis of the distribution of vascular disease of the brain; and in some regards they may be compared to the cerebral manifestations which sometimes mark the acute phase of myocardial infarction (6).

One of the notable features of congestive failure in these patients was the lack of response to the usual cardiac remedies, notably digitalis, diuretics and oxygen. There were 17 patients whose heart failure was rapidly progressive and who died after all forms of treatment had failed. In 9 others there was an early response to treatment, but without obvious reason the course then took a turn for the worse and then treatment was without manifest effect. Thus 26 of the 78 cardiac patients died after a period where the usual therapy for heart failure was not successful. These cases were proportionally distributed among those with severe, moderate and mild stenosis.

In 15 cardiac patients death followed a period of coma. Here also the degree of stenosis was distributed in proportion to the total number of cases. Coma also appeared toward the end of the disease in 8 patients of the non-cardiac group.

The group of 22 patients whose end came with sudden death will be discussed later.

In the 7 remaining cardiac patients death was precipitated by pulmonary infarction, pneumonia or myocardial infarction.

Discussion: Since this study is based on autopsied cases the hospital course had an inevitable outcome, progressing rapidly or slowly, steadily or episodically to death. The symptomatology was chiefly that of heart failure of the congestive

or forward type or both combined. One of the features of the hospital course was a crisis of sweating, cyanosis, restlessness and confusion, usually with flushing but sometimes with pallor. Often the onset was abrupt and without an obvious precipitating cause; and the recovery was gradual in most cases, rapid in a few. The drenching sweat was a prominent feature. Such spells were not relieved by any of the therapeutic measures exhibited. They were more severe and more frequent in cardiacs with advanced stages of stenosis. We have interpreted the attack as a manifestation of acute forward failure of the heart, and emphasize it as an omen of grave import, since it appeared only rarely in patients who recovered and were discharged from the hospital improved.

Cardiac pain occurred in the hospital in only 9 of the 40 patients who had suffered it prior to admission. This is in keeping with the observation that pain was often related to exertion although pain itself usually was delayed and did not interdict activity as it may in ordinary angina pectoris.

Mental symptoms, convulsions and syncope marked the course in another group of patients, and sometimes they were associated with the crises described above. The localization of signs was erratic from patient to patient but in an individual tended to conform to a given type. This suggests that forward failure is the primary cause but the clinical pattern depended upon vascular disease of the brain.

XIII. TREATMENT

A review of the cases in this series repeatedly gives testimony to the difficulty of therapy and the poor or atypical response to the measures used in treatment. Digitalis was used often and the response to it was singularly poor. Tachycardia in particular responded poorly to digitalis. Patients who recovered from a definite bout of congestive failure usually did so only after full digitalization had been established for considerable periods. There was not the decided improvement which often follows quickly upon adequate digitalization in the presence of congestive heart failure. There was no indication that digitalis had any toxic effect—rather it seemed ineffectual in helping the ventricle overcome the load imposed by the rigid obstruction at the aortic valve.

The employment of diuretics, especially those containing mercury, was little more successful, although in a few instances definite clinical improvement followed induced diuresis. Oxygen was used frequently and it was sometimes very helpful but in the majority of instances there was a discouraging lack of response. Restriction of salt, although employed in but a few patients, was not especially satisfactory. If diuresis was established, there was generally a definite improvement. Bed rest is difficult to evaluate as a separate factor, since all patients were treated in bed. There was no discernible difference, among patients with more than one admission, in the course of those with long rest and those who were ambulatory early. Rest as a prophylactic measure cannot be evaluated. While the data can hardly be considered conclusive, patients who lived within their known exercise tolerance fared better than those who did not. There was some indication that this was more helpful in preventing congestive failure than in

reducing the number of syncopal attacks or other indications of forward failure. Treatment of syncope, shock and the hypokinetic syndrome was various in its method, constant in its ineffectiveness. Heroic therapy was no better than none at all. Trial of the usual measures for medical shock was uniformly disappointing. Oxygen was not very helpful.

In summary, the course of the disease was influenced very little by the usual forms of cardiac treatment. The mechanical obstruction, which kept a strain on the left ventricle, was not available for physical correction. When an added call for work fell upon the ventricle already burdened by working at maximal capacity to satisfy basal needs, failure resulted. The hypertrophied and sometimes dilated ventricle was helped very little by digitalis and the general state not much improved by other agents. Recovery from a bout of failure seemed to depend on the capacity to muster some hidden reserve, an innate function inaccessible to the ordinary forms of therapy. When it failed, death ensued.

XIV. CARDIAC PAIN

In numerous articles on aortic stenosis reference is made to cardiac pain and almost always the term *angina pectoris* is used to describe it (10, 17, 21, 28, 55, 82). We have studied the various types of pain noted by patients in this series and have separated the cases with cardiac pain for comparison with the whole group (see Table 15). Cardiac pain occurred in 40 of the 107 cases, an incidence of 37%. A description of the pain was available in 32 cases; in the remaining 8 there was merely mention that pain had been experienced, but no description recorded. In 12 cases the pain was substernal in locus, oppressive, constricting, gripping or described as a sense of fullness or tightness. There was no radiation. A strict relation of effort was not observed but in a number of cases pain occurred a little while after some untoward exertion such as running for a car or heavy lifting. The pain usually did not check activity but occurred a few minutes to an hour after exercise was completed. Non-radiating epigastric pain occurred in one, and in 2 epigastric pain spread up to the retrosternal area. In 4 the pain was most pronounced in the anterior aspect of the right chest. Pain beginning in the right side of the chest radiated to the right shoulder and arm in another patient and to the right shoulder alone in still another. The remainder of the locations are listed on Table 15. There was but one case of classical *angina pectoris*.

Age, sex, color, medical history, physical examination, response to treatment and course of the disease did not show significant discrepancies between those who had pain and those who did not. Therefore our attention was drawn to a study of the morphology of the heart. A primary question concerns the possible rôle of coronary artery disease and myocardial infarction, another the rôle of different degrees of aortic stenosis in causing pain.

In Table 15 we have a comparison of the coronary sclerosis in those with cardiac pain and the entire group. Though the significance of small numbers of cases is not great, there was definitely less coronary sclerosis of advanced grades in those with cardiac pain than in the whole group. The pathologic data, therefore, pro-

vide nothing against the concept that the pain in victims of aortic stenosis is basically different from that of true angina. Cardiac ischemia, however, is not exonerated by this evidence. The percentage of cases with advanced or moderate aortic stenosis was 95 in those with pain and 80 in those with no pain. Other relationships are given in Table 16.

Discussion: We have studied the problem of cardiac pain in aortic stenosis with care since many authors have used the term angina pectoris to describe it. While the definition of a symptom complex such as angina pectoris is elastic, it has been used somewhat uncritically in association with the pain in aortic stenosis. If by

TABLE 15
Cardiac pain

LOCATION	TYPE	RADIATION	NO. OF CASES
Not Described	—	—	8
Substernal	Oppressive Tightness	0	12
Epigastric	Oppressive Tightness	0	1
Epigastric	Oppressive Tightness	Sternum	2
Epigastric	Oppressive Tightness	Left Chest	1
Precordial	Oppressive Tightness	0	6
Left Chest	Oppressive Tightness	0	1
Right Chest	Oppressive Tightness	0	4
Right Chest	Oppressive Tightness	Right Shoulder	1
Right Chest	Oppressive Tightness	Right Shoulder and Right Arm	1
Only between Shoulder Blades	Stabbing	0	1
Around Umbilicus	Sharp	0	1
Typical Angina		Left Anterior Shoulder	1
Total.....			40

	DEGREE OF CORONARY SCLEROSIS				MYOCARDIAL INFARCTS	
	Absent or Minimal	Mild	Moderate	Severe	Old	Recent
Cardiac Pain Group.....	12%	46%	15%	29%	10%	7%
Entire Group.....	15%	36%	24%	25%	16%	18%

	DEGREE OF AORTIC STENOSIS	
	Those with Cardiac Pain	Those with No Pain
Severe and Moderate.....	95%	80%
Mild.....	5%	20%

angina one means a transitory substernal or precordial pain, constricting in character, usually radiating down the ulnar aspect of the left arm, provoked by exercise, emotion or exposure to cold, then angina pectoris was described by only one of our patients. The relation to exercise was usually that of a delayed reaction rather than an immediate response, the pain lasted longer and was not characteristically moderated by nitroglycerin. Study of the hearts in subjects with cardiac pain revealed that there was less coronary sclerosis than in the whole group. The severity of the aortic stenosis, however, was generally great. It must be remembered that angina pectoris is uncommon in patients seen in the

clinic or on the wards of this hospital. Cardiac pain in our patients with aortic stenosis differed from that described in the angina of coronary arteriosclerosis. It is possible that no useful purpose is served by adherence to so strict a definition of angina pectoris, though other observers have noted that pain in aortic stenosis may not be typically anginal in character (68). We have no explanation of radiation to the right instead of to the left.

XV. SUDDEN DEATH

The problem of sudden death is confused by a failure of most authors who discuss this topic to define what they mean by sudden (12, 25, 35). Included in the

TABLE 16

Correlation of old and recent infarcts with stenosis, coronary arteriosclerosis and cardiac pain

CASE	DEGREE OF STENOSIS	OLD INFARCT	NEW INFARCT	CORONARY ARTERIO-SCLEROSIS	CARDIAC PAIN
7	++	0	2 areas L.V.	+	0
10	++	septal, moderate	0	++	0
18	+	many small	0	++	0
19	+++	many small	0	+++	0
28	++	many small	0	+++	+
34	++	0	mod. septal	+++	0
35	++	many small	many small	++	0
30	+++	apex 2 x 3 cm.	0	+	+
38	+	many small 2 cm.	0	++	0
40	+++	0	many small	++	+
44	+++	many small	apex 2 x 2 cm.	++	0
48	+	0	apex 2 x 2 cm.	+	0
60	++*	0	small embol SBE	+	0
53	++	0	mod. L.V.	+	0
62	+++	many small	0	++	+
64	++	many small	lat. L.V. 3 x 5 cm.	+++	+
72	++	0	mod. apex	+++	0
74	+++	many small	0	+++	0
76	++	0	mod. apex	+	+
78	++	many small	0	+	+
85	++	0	rt. vent. mod.	+++	0
106	+++	L.V. post 2 x 2 cm.	0	+	+
107	+	large septal	large lat. L.V.	+++	+

* Group II.

medical literature under the term sudden death are cases which fall into three broad categories. These include instant death, the sudden unheralded syncopeal deaths where there is an abrupt cessation of vital functions. It may commence as an ordinary fainting spell but from it there is no recovery (5, 39, 80). It is especially frequent among persons suffering from angina pectoris and coronary thrombosis.

A second form of sudden death differs from the first mainly in the duration of the agonal scene. From the onset of the terminal stages until death, minutes elapse, usually between 5 and 30, during which the patient goes through a series of phenomena which indicate that asystole or its physiologic counterpart, ventricular fibrillation, has occurred. On examination, it one chances to be present at

the terminal episode, the heart is silent—only occasional muffled beats are heard or no sounds at all. Severe respiratory stress is usually evident, air hunger, violent gasps and great efforts to breathe are characteristic. Cyanosis usually occurs and deepens rapidly. Profuse sweating may appear. Maniacal thrashing about may also be seen, though this is not constant. The level of consciousness is sometimes impaired though apparently in many cases the victim senses the urgency of the catastrophe and may call out for help. In a good many such patients there is a grim fear of impending dissolution akin to the *angor animi* of angina pectoris, though by the nature of events an adequate description of sensations is rarely obtained. Convulsions sometimes happen, and may be focal though more often they are general and tonic. After a period of minutes the struggling ceases, gasps become more widely spaced and at length all respiratory effort ceases. The heart, quiet to auscultation, may show bizarre arrhythmias if electrographic tracings are made; and there is generally indication of electrical activity for some moments after all sounds of cardiac action have ceased. Many types of stimuli may precipitate this manner of dying. It is relatively common.

The third category of sudden death is really a conglomerate assembly of conditions which have little in common save the fact that death at the particular time of its occurrence was not expected. It is really not so much sudden as unanticipated. Included in this group are cases which "went bad" but where survival may have lasted up to 24 hours. Actually most of the deaths which occur from cerebral accidents, pulmonary infarcts and emboli generally, and which are called *sudden deaths*, fall into this group. Life may persist for hours but with the gradual alteration of vital functions life ebbs out slowly after the forces of dissolution have set in. Much of the medical literature on sudden death deals with this general type of dying. Certainly this is the class of sudden death which has tintured folklore, has given poets and theologians themes for their outpourings and which often enough figures in the lay press, ordinarily more attuned to battle, murder and accident.

Insofar as our data give evidence, the sudden death in these hospitalized patients suffering from aortic stenosis is a matter of minutes—not the irrevocable syncope of those who literally drop dead, nor the slower falling away of those whose lethal scene is measured in hours. This type of sudden death was encountered 22 times in this series; and for the most part an essentially regular chain of events occupied the terminal minutes of life. We have separated these cases from the whole group to see whether there was a peculiar clinical or morphological basis which might serve as an omen to assist us in prognosis, or as an explanation in terms of morphology. This search has been unfruitful on the clinical side but the extent of the pathological findings was consistently great. There was no significant difference from the whole series in natural history: age, sex and color did not show any trend away from the distribution of the entire group. There were 16 white males, 4 white females and 2 colored males. The duration of symptoms of the disease in those who died suddenly ranged from more than 11 years to a few weeks and did not differ markedly from the duration of the group as a whole.

Since all the patients who died suddenly were in the cardiac group, and all but

one had severe or moderate degrees of stenosis, we have compared the findings in this sub-group with the entire group of cardiac cases in Table 17. The symptoms followed the same trend as those in the cardiac group, though patients who died suddenly had a higher incidence and greater severity of dyspnea (100 and 93 percent) and dizziness (45 and 32 percent), the same incidence of cardiac pain (50 and 50 percent) while edema was less common (55 and 73 percent). Nothing in the clinical course was of great predictive value in warning that a particular patient was liable to sudden death. It is of significance that all patients who died suddenly were in Group I. They were all recognized as suffering from heart failure. Syncope was not a common forerunner of this mode of death, occurring in 9 percent of the cases compared to 17 percent of the whole group. This is a different state of affairs from the instant, physiologic death which may end the

TABLE 17
Comparison of Group I cases and victims of sudden death

	SUDDEN DEATH	GROUP I
Number	22	78
Heart Enlarged	94%	78%
S ₁ Apex Clear	33%	20%
Muffled	67%	50%
S ₁ Apex Clear	50%	24%
Muffled	50%	76%
S ₂ Base Muffled	75%	42%
Absent	25%	58%
A ₂ > P ₂	25%	34%
A ₂ = P ₂	25%	20%
A ₂ < P ₂	50%	46%
Murmurs		
Systolic	73%	86%
Apical Diastolic	18%	30%
Basal Systolic	86%	88%
Diastolic	37%	37%
Thrill	41%	23%
Degree of Stenosis		
Severe	45%	47%
Moderate	50%	46%
Mild	5%	7%

life of victims of angina pectoris or infarction of the heart where a history of syncope attacks is more common in those who experience instant death than in those who do not (5). This negative history in our patients who died suddenly is surprising, since syncope is one of the clinical landmarks in patients with aortic stenosis, and we had expected to find it very frequent in those who died suddenly. This fact may be additional testimony that instant death (the lethal crisis measured in seconds) is not the same phenomenon, and has a different mechanism from sudden death where the final episode is measured in minutes. The observations of Marvin and Sullivan on sudden death in *ambulatory* patients with aortic stenosis indicate that it may be really instant death, perhaps associated with arrhythmias. Their suggestion of a relation to carotid sinus sensitivity is not convincing and we had no indication in our cases that a hyperactive carotid sinus was important in either syncope or sudden death.

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Murmurs		
Systolic	73%	86%
Diastolic	18%	30%
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Severe	45%	47%
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Causes of Death: In addition to the striking examples of sudden death, other modes of death and causes of death are detailed in Table 18. Group I patients all died for reasons directly or closely associated with the aortic stenosis. The Group II patients, except for those with bacterial endocarditis and injury following syncope, died of unrelated causes which are listed in order of frequency. In the majority the aortic stenosis had been latent symptomatically or produced only minor trouble.

XVI. ACCURACY OF DIAGNOSIS

The accuracy of diagnosis of aortic stenosis in our series of cases is a reflection of past teachings in demanding classical findings before the diagnosis was con-

TABLE 18
Type of death (Group I)

Sudden.....	22
Coma.....	15
Progressive Congestive Failure with No Response to Treatment.....	17
Congestive Failure after Initial Response to Treatment.....	23
Hemiplegia.....	1
	78

Cause of death (Group II)

DEGREE OF STENOSIS.....	SEVERE	MODERATE	MILD	TOTAL
Bacterial Endocarditis.....	4	3	1	8
Carcinoma.....	3	2	2	7
Peptic Ulcer.....	2	1	0	3
Subdural Hematoma.....	0	1	1	2
Cerebral Hemorrhage.....	0	1	1	2
Pneumonia.....	0	0	2	2
Meningitis.....	1	0	0	1
Poison.....	0	1	0	1
Trauma.....	0	0	1	1
Tuberculosis.....	0	0	1	1
Genito-urinary Infection.....	0	0	1	1
Total.....				29

sidered. The consulting staff made the correct diagnosis in 11 of 39 cases seen, approximately 28 percent. Seven of these cases had severe and 4 moderate stenosis. The house staff made a correct diagnosis 26 times, approximately 24 percent of all cases. Thirteen of these had severe stenosis, 12 moderate, one mild. Both aortic and mitral stenosis were thought present by the consulting staff in 15 percent of cases and by the house staff in 9 percent. A mitral valvular lesion only was diagnosed by the consulting staff in 5 percent of cases and by the house staff in 14 percent. Luetic aortic valvulitis was thought present by the consultants in 8 percent of cases and by the house staff in 6 percent. The diagnosis of hypertensive or coronary artery disease or both was made by the consulting staff in 33 percent of the patients and by the house staff in 31 percent. Recent myocardial infarction was the diagnosis by the consulting staff in 5 percent while the

house staff thought 6 percent of the cases had acute myocardial infarction but such a lesion was not found at autopsy in any case so diagnosed, and it was not diagnosed correctly in any of the cases where it was found (56).

The greatest accuracy of diagnosis was found in Group I patients with severe or moderate aortic stenosis. In cardiacs with severe stenosis, the consulting staff made a correct diagnosis in 7 of 16 cases seen (43 percent); the house staff in 33 percent. The consultants were accurate in 25 percent of the cardiac cases who had moderate aortic stenosis and the house staff in 27 percent. No cardiac case with mild aortic stenosis was correctly diagnosed.

XVII. PATHOLOGIC DATA

The basis for classifying the extent of stenosis in the three groups of severe, moderate and mild and for the clinical division into Group I and Group II was given on p. 3. The degree of stenosis by sex and color is found in Figure 7.

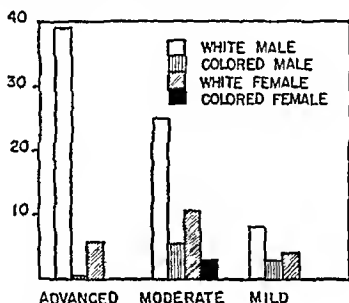


FIG. 7. DEGREE OF STENOSIS (AUTOPSY)
Distribution of degrees of stenosis by color and sex.

Severe stenosis occurred in 44 percent, moderate in 42 percent and mild in 14 percent. Fibrosis and calcification existed in all aortic valves and the amount of calcium was closely related to the extent of stenosis. Sometimes calcification extended into the ventricular wall and occupied part of the base of the aortic leaf of the mitral valve without impairing its normal mobility. When stenosis was severe or moderate the sinuses of Valsalva were extensively calcified in most instances. In 8 cases there was complete calcification of the aortic ring.

Fusion of cusps existed in 47 cases (44 percent) and varied from a partial joining at the base to total fusion of two cusps along the whole of their adjacent surface. Fusion, especially when extensive, naturally produced extreme degrees of stenosis. It did not occur with mild stenosis. In 2 cases (cardiacs) with severe stenosis the partial fusion was judged to indicate a congenitally bicuspid valve (42, 44).

Nodule formation occurred in 32 cases (30 percent) in only one of which was stenosis mild. The nodules ranged from 1 to 8 mm. in diameter; and were found

on aortic and ventricular aspects of the valve.^{ad} The degree of nodularity varied from well formed discrete nodules to less distinct forms blending into plaque-like or diffuse calcification. In 7 cases (6 percent) nodules were confined to the line of valve closure.

Atheromatous ulcers of a non-bacterial nature were found on the aortic valves in 6 cases. Eight of the cases in Group II had stenosis with active vegetative lesions of bacterial endocarditis; and in another a band of vegetations 2 cm. thick completely encircled the aorta just above the insertion of the valves.

In 5 cases (2 were Group II with mild stenosis) the free edges of the aortic valves were rolled extensively but there was no evidence of syphilis.

Since care was taken to exclude cases with what might be construed as valvular lesions impairing the function in valves other than the aortic, description of the mitral, tricuspid and pulmonary valves can be perfunctory. In 15 cases (14 percent) with normal chordae tendineae one or both mitral valves were slightly but definitely thickened. Sclerotic plaques appeared at the bases of the mitral leaflets in 16 cases (15 percent). In 9 cases a few small fibrous nodules were present along the edges of the mitral leaflets. In 3 cases with bacterial endocarditis minute vegetations were found on the edge of the mitral leaflets. Partial calcification of the mitral ring was noted twice. Seven cases had sclerotic plaques in the base of the tricuspid valves; one had slight tricuspid valve thickening and another a few nodules along its free edge. Sclerotic plaques at the base of the pulmonic valves occurred in 4 cases and thickening of normally functioning pulmonic valves was found once.

Figure 8 shows the incidence of the various heart weights. The heaviest hearts were those of males. Table 19 shows the incidence of the various heart weights for Group I and Group II cases based on the degree of aortic stenosis. The mean heart weights varied directly with the degree of stenosis and the average for each class of cardiacs was greater than that for any class of non-cardiacs. The average weight for the cardiac patients with severe aortic stenosis was 598 grams; for moderate stenosis 530 grams; for mild stenosis 518 grams. Average weights for the non-cardiac patients with severe stenosis were 495 grams; with moderate stenosis 446 grams; and with mild stenosis 351 grams. The heaviest heart, that of a patient with bacterial endocarditis, weighed 860 grams. The lightest heart weighed 135 grams and was found in a 38 year old white woman who died of carcinoma of the cervix and was found to have mild aortic stenosis. The extreme weights occurred in patients who had not had clinical evidence of heart failure.

Left ventricular measurements varied in the same manner as heart weights with one exception; those of non-cardiac patients with severe stenosis slightly exceeded cardiac cases with mild stenosis (see Table 19). No striking differences were noted in right ventricular measurements.

Seventy hearts were thought to show dilatation at autopsy and there was no relationship to the degree of stenosis or of hypertrophy of the myocardium.

Auricular thrombi were found only in cardiac cases with severe or moderate aortic stenosis. Six cases had thrombi in the right auricle only, one in the left and 5 in both.

Pericardial milk spots were found in 20 cases, 15 of these being cardiacs. A Group I case with severe stenosis had fibrinous pericarditis. One cardiac patient

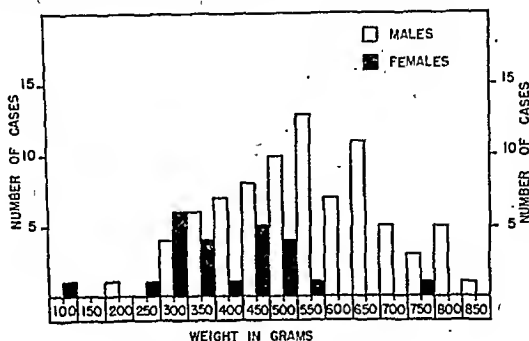


FIG. 8. HEART WEIGHT
Distribution of heart weights by sex.

TABLE 19
Heart weights

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Weight in Grams:							
135-149.....	0	0	0	0	0	1	1
150-199.....	0	0	0	0	0	0	0
200-249.....	0	0	0	0	0	1	1
250-299.....	0	1	0	0	0	0	1
300-349.....	1	2	0	2	3	2	10
350-399.....	2	2	2	1	1	2	10
400-499.....	1	5	0	2	0	0	8
450-499.....	3	5	0	2	1	2	13
500-549.....	5	4	1	0	3	1	14
550-599.....	7	7	0	1	0	0	15
600-649.....	3	4	0	0	0	0	7
650-699.....	5	3	2	0	1	0	11
700-749.....	5	0	0	0	0	0	5
750-799.....	2	1	0	1	0	0	4
800-849.....	3	2	0	0	0	0	5
850-899.....	0	0	0	1	0	0	1
Unknown.....	0	0	0	0	0	1	1
							107
Average.....	598	530	518	495	448	351	
Average Thickness of Left Vent. (mm.).....	18	17	16.6	17.2	16	15	
Average Thickness of Right Vent.....	5.2	4.8	4.6	4.4	5	4.3	

with mild stenosis had moderate adhesive pericarditis. Pericardial fluid varying from 50 to 250 cc. was found 17 times, only in those classified as cardiacs.

It has been said (28) that sclerosis of the aorta and coronary vessels is likely to occur in inverse proportion to the degree of stenosis of the aortic valve and that acute coronary occlusion with myocardial infarction is uncommon in aortic stenosis. The pathologic findings in the hearts of our 107 cases do not bear out this opinion (see Tables 20 & 21). Thirty-one cases had severe coronary sclerosis, 23 had moderate coronary sclerosis and 46 had definite but mild sclerosis. No evi-

TABLE 20
Old and recent infarcts with degree of stenosis and coronary atherosclerosis

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Coronary Sclerosis +++.....	8	14	2	2	3	2	31
++.....	6	9	1	2	1	4	23
+.....	20	14	2	4	4	2	46
0.....	2	0	0	0	1	2	5
Old Infarct.....	6	5	2	0	0	1	14
Recent Infarct.....	2	9	2	0	0	0	13
Myocardial Fibrosis ++++.....	7	6	0	0	1	0	14
++.....	8	15	2	6	2	4	37
+.....	14	10	2	1	3	4	34
0.....	7	4	1	3	3	2	20

TABLE 21
Degree of arteriosclerosis of aorta

Degree of Stenosis.....	GROUP I			GROUP II			TOTAL
	Severe	Moderate	Mild	Severe	Moderate	Mild	
Arteriosclerosis of Aorta:							
Ascending +++.....	0	0	0	0	0	0	0
++.....	1	6	1	2	0	0	10
+.....	14	14	1	1	2	6	38
0.....	21	17	3	7	7	4	59
Thoracic +++.....	7	13	1	1	2	0	24
++.....	13	12	2	3	1	6	37
+.....	13	10	2	2	4	2	33
0.....	3	2	0	4	2	2	13
Abdominal +++.....	12	19	2	3	3	5	44
++.....	10	11	3	2	2	3	31
+.....	14	4	0	3	4	2	27
0.....	0	3	0	2	0	0	5

dence of coronary sclerosis was found in 2 cardiac patients who had severe aortic stenosis; in one non-cardiac patient who had moderate aortic stenosis; and in 2 non-cardiac cases who had mild aortic stenosis. Extreme coronary ostial narrowing caused by distortion from the aortic lesion was seen in 13: in the cardiac group 10 had severe aortic stenosis, 2 moderate and 1 mild.

Scars of old myocardial infarctions were present in 14 cases (13 percent of total) and 13 of these were cardiac patients. Of these 13 cardiac cases (17 percent of all cardiacs) 6 had severe stenosis, 5 moderate and 2 mild (see Table 20). The

one non-cardiac case with old myocardial infarction had mild aortic stenosis. Of these 14 cases, 10 had anterior left ventricular scars. The size of the old myocardial infarcts varied but was not related strictly to the degree of aortic stenosis or of coronary atherosclerosis. Of the 6 cardiac patients with severe aortic stenosis, 4 had "several small" healed infarcts (up to 1 cm. in diameter). Of these 4, two had severe coronary atheroma and 2 moderate. The other 2 cases had the largest old infarcts (2 x 3 cm; 2 x 2 cm.) and each had only mild coronary atherosclerosis. Of the 5 cardiac patients with moderate aortic stenosis who had old myocardial infarcts, 4 had several small lesions with coronary sclerosis severe in two, moderate in one and mild in the last. The remaining case had an old septal infarct with only mild coronary sclerosis. Of the two cardiac cases with mild aortic stenosis who had old myocardial infarcts, one had many small lesions with moderate coronary atherosclerosis; the other exhibited a large septal scar and revealed severe coronary sclerosis. The remaining non-cardiac case with mild aortic stenosis had many small healed infarcts in addition to moderate coronary atherosclerosis. Recent myocardial infarction was found in 13 cases (12 percent of total) and all except one of these were cardiac cases (15 percent of the cardiac cases). Two had severe stenosis, 9 moderate and 2 mild. There was anterior left ventricular involvement in 9 of these 12 cases. A mural thrombus was present in 4.

The size of the recent myocardial infarcts varied and, just as with the old ones, did not show a direct relationship to the degree of aortic stenosis or of coronary sclerosis. Of 2 cardiac patients with severe aortic stenosis, one had many small recent myocardial infarcts; the other a recent one at the apex measuring 2 x 2 cm. Both had moderate coronary atherosclerosis. Nine cardiac patients with moderate aortic stenosis showed recent myocardial infarcts. In six of these the lesions were of moderate size: 2 apical, 2 of the lateral wall left ventricle, 1 of the right ventricle and 1 of the septal wall. Of these six cases, 3 had severe coronary atherosclerosis and 3 mild. Of the 3 others one had many small recent infarcts with moderate sclerosis of coronary vessels; another a large (3 x 5 cm.) fresh lesion of the left ventricle with severe coronary sclerosis; the last was a non-cardiac case showing many small infarcts due to emboli from an aortic valvular subacute bacterial endocarditis. Two cardiac cases with mild aortic stenosis exhibited recent myocardial infarcts. In one the moderate sized lesion at the apex was associated with mild coronary atherosclerosis; in the other a large left ventricular lesion was found with severe coronary atheroma. Recent myocardial infarction was not found in any Group II case except as noted.

Advanced diffuse fibrosis of the ventricles was present in 14 cases; 7 with severe stenosis and 7 with moderate. Moderate diffuse fibrosis was present in 37 cases: 14 with severe stenosis, 17 moderate and 6 mild. Mild diffuse fibrosis was present in 34 cases and 15 of these had severe stenosis, 13 moderate and 6 mild. No ventricular fibrosis was recorded in 20 cases: 10 with severe stenosis, 7 with moderate and 3 with mild.

No case showed severe sclerosis of the ascending aorta, but moderate atherosclerosis was present in 10 cases; 3 of these had severe aortic stenosis, 6 moderate

and 1 mild. Mild atherosclerosis was present in 38 cases. The degree of atheromatous change in the ascending aorta was in marked contrast to the findings in the descending aorta where the abdominal portion was most seriously diseased. In the thoracic portion of the descending aorta, severe atherosclerosis was seen in 24 cases; 8 of these had severe aortic stenosis, 15 moderate and 1 mild. In 14 of these 24 cases ulceration was seen with thrombus formation in 2 cases. Moderate atherosclerosis was present in 37 cases and 16 of these had severe aortic stenosis, 13 moderate and 8 mild. Mild atherosclerosis was present in 33 cases; 15 had severe stenosis, 14 moderate and 4 mild. Severe atherosclerosis of the abdominal aorta was present in 43 cases. Ulceration was apparent in 36 of these with thrombus formation in 11. Of these 43 cases, 15 had severe aortic stenosis, 22 had moderate and 6 mild. Moderate atherosclerosis was present in 31 cases of which 12 had severe aortic stenosis, 13 moderate and 6 mild. Mild atherosclerosis was found in 27 cases and 17 of these had severe aortic stenosis, 8 moderate and 2 mild. The degree of aortic stenosis had no direct relationship to the degree of atheromatous change in the descending aorta (see Table 21).

Accumulation of fluid in body cavities as the result of congestive failure was found in many cases at autopsy; mainly in cardiac patients with severe or moderate aortic stenosis. Bilateral hydrothorax was present in 47 cases. The amount varied from 150 cc. to 4000 cc. on each side. In 28 cases the amount present was greater on the right than left; greater on the left than right in 7; equal on both sides in 12 cases. Of these 47 cases the clinical diagnosis was correct in 13 cases. Of those who had x-rays of the chest, fluid was found in 12 of 33 cases.

Right hydrothorax only was present at autopsy in 9 cases and 5 of these were diagnosed clinically. By x-ray examination the diagnosis was made in 3 cases. In 2 cases the left pleural cavity was obliterated. In 1 case 2300 cc. of fluid were present in the right pleural cavity and the left cavity appeared normal. Hydrothorax of the left side only was present in 5 cases. The amount of fluid varied from 100 to 1200 cc. In all of these cases the pleural cavity on the right was obliterated by adhesions.

Seven cases had hydrothorax and ascites; 6 cases had hydrothorax and pericardial effusion; 11 cases had hydrothorax, ascites and pericardial effusion. Ascites was present in 18 cases and a correct clinical diagnosis was made in 10. In 3 cases ascites was thought present clinically but at autopsy none was present.

Pericardial effusion varying from 30 to 250 cc. occurred in 16 cases. The clinical diagnosis was correct in 1 case (250 cc.).

Pulmonary infarction was found at autopsy in 11 cases. Seven of these had moderate aortic stenosis and 4 severe aortic stenosis. Of these 11 cases, 10 were found in cardiac cases and one in a non-cardiac (subacute bacterial endocarditis). Five of these patients had auricular thrombi; bilateral in 4 and right sided in the other. One case had bilateral mural thrombi at the apex of each ventricle (recent myocardial infarction). Five cases did not have mural thrombi. The infarcts were multiple and bilateral in 8 cases; in the left lower lobe in 2 cases; the last case revealed complete occlusion of the right pulmonary artery by a large embolus broken off from a vessel in the leg.

and 1 mild. Mild atherosclerosis was present in 38 cases. The degree of atheromatous change in the ascending aorta was in marked contrast to the findings in the descending aorta where the abdominal portion was most seriously diseased. In the thoracic portion of the descending aorta, severe atherosclerosis was seen in 24 cases; 8 of these had severe aortic stenosis, 15 moderate and 1 mild. In 14 of these 24 cases ulceration was seen with thrombus formation in 2 cases. Moderate atherosclerosis was present in 37 cases and 16 of these had severe aortic stenosis, 13 moderate and 8 mild. Mild atherosclerosis was present in 33 cases; 15 had severe stenosis, 14 moderate and 4 mild. Severe atherosclerosis of the abdominal aorta was present in 43 cases. Ulceration was apparent in 36 of these with thrombus formation in 11. Of these 43 cases, 15 had severe aortic stenosis, 22 had moderate and 6 mild. Moderate atherosclerosis was present in 31 cases of which 12 had severe aortic stenosis, 13 moderate and 6 mild. Mild atherosclerosis was found in 27 cases and 17 of these had severe aortic stenosis, 8 moderate and 2 mild. The degree of aortic stenosis had no direct relationship to the degree of atheromatous change in the descending aorta (see Table 21).

Accumulation of fluid in body cavities as the result of congestive failure was found in many cases at autopsy; mainly in cardiac patients with severe or moderate aortic stenosis. Bilateral hydrothorax was present in 47 cases. The amount varied from 150 cc. to 4000 cc. on each side. In 28 cases the amount present was greater on the right than left; greater on the left than right in 7; equal on both sides in 12 cases. Of these 47 cases the clinical diagnosis was correct in 13 cases. Of those who had x-rays of the chest, fluid was found in 12 of 33 cases.

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Forty-one cases had chronic passive congestion of the kidneys. Arterionephrosclerosis occurred in 7 and pyelonephritis in 6. Multiple renal infarcts were found in 5 and focal embolic glomerulonephritis in 5. Severe toxic nephrosis was found twice and renal lithiasis and multiple tumor metastases once each.

A wide range of abnormal findings not related to the cardiovascular system was found at autopsy, mainly in the non-cardiac group of cases. Nine cases had some type of carcinoma, 13 cases had cholelithiasis, 1 case had acute necrotizing hepatitis, 3 cases had cirrhosis of the liver. Two cases were found with adrenal cortical adenomata and 2 others disclosed non-toxic adenomata of the thyroid. The cause of death in 3 cases was a bleeding duodenal ulcer. Two patients had fractured hips. One patient had a thrombophlebitis of the left common iliac vein.

Several kinds of intracranial lesions were found at autopsy. One case had an aneurysm of a cerebral artery; another was found to have an 8th nerve tumor. One patient died because of a purulent meningitis. One patient had a skull fracture and subdural hematoma as a result of an automobile accident. Two other cases died because of undiagnosed subdural hematomata. It is of interest that both of these cases, as well as the two cases with fractured hips, had vertigo as one of the symptoms of aortic stenosis, and their accidents were thus attributable to the valvular disease.

Discussion: There was a close relationship between average heart weight and degree of stenosis; and this prevailed in those who had congestive failure and those who did not. The cardiaes all had heavier average heart weights than did those with advanced stenosis who had not had congestive failure. The thickness of the left ventricle followed much the same trend. Contrary to previous reports coronary arteriosclerosis was prevalent though its extent had no striking association with that of the aortic stenosis. It was sufficiently extensive to have produced myocardial fibrosis or coronary thrombosis in some cases.

The findings at autopsy were influenced by the selection which weeded out all cases where complicating disease of mitral or other valve might have produced a physiologic disturbance, though there were mild lesions in the mitral valve in a number of cases. Calcification, gross or microscopic, existed in every stenotic aortic valve. When the obstruction was advanced the sinuses of Valsalva were involved, and in a few instances there was complete calcification of the aortic ring. Nodules were found in association with extreme degrees of stenosis, and fusion of the cusps occurred in nearly half of the cases. In 2 cases the fusion was thought to be congenital, giving rise to a bicuspid valve. The superimposed lesions of bacterial endocarditis occurred in 8 cases and a thick band of bacterial vegetations surrounded the aorta just above the valve in another case. In 6 instances there were atheromatous ulcers of a non-bacterial type

XVIII. COMMENT

Analysis of our data on 107 cases of pure aortic stenosis has verified many of the general teachings regarding it, contravened others and cast light on some still controversial aspects of the disease. It leaves many questions without final

answer. Since our figures are larger than groups reported heretofore, and since the selection of cases eliminated complicating valvular lesions the observations deserve emphasis. In a careful search of much of the modern medical writing on the subject we did not uncover any series of cases, chosen to exclude other valve disease, which was of comparable size, or based on autopsied cases only. Indeed several of the widely quoted studies deal with clinical cases only, or with series of clinical and postmortem cases combined. Frequently the criteria for diagnosis reduce the usefulness of the clinical data by defining the disease in terms of diagnostic requirements and then saying that aortic stenosis is characterized by the signs found in patients selected on this basis. Medical and cardiac textbooks have been confirmed offenders and by repeating the dicta of some predecessor, permit the picture of disease to become fossilized in simple but inadequate terms which become established by reiteration. A fresh approach based on proved cases facilitates the reappraisal of the problem in more than just descriptive terms. For these reasons we have presented our findings in detail and have not included a review of the medical literature.

In terms of the diagnostic triad of basal systolic murmur transmitted upward, thrill and small slowly rising pulse our cases departed notably from the textbook. Only slightly more than a fifth satisfied even the first and second criteria. It should be stressed that in the presence of overwhelming failure or shock the murmurs are often absent, even where they have been noted previously and when they appear later upon improvement.

XIX. SUMMARY AND CONCLUSIONS

1. A series of 107 proved cases of aortic stenosis, uncomplicated by deforming lesions of other valves, has been studied from the clinical and morphological aspects.

2. Aortic stenosis found at autopsy is preponderantly a disease of the late years of life although some cases occur in the early decades. Roughly three-fourths of the cases were males. Color, anthropologic type and occupation played no significant rôle in the disease.

3. A history of acute rheumatic fever was obtained in two-thirds of the cases with relevant data.

4. Cases were divided into "cardiac" (Group I) and "non-cardiac" (Group II) on the basis of history and status on hospital admission. Of the 78 cardiacs, 34 had chronic congestive failure, 10 had intermittent bouts of failure and 19 had an abrupt onset of failure shortly before they were admitted. Severe nosebleeds precipitated failure in 3 cases. Five patients with congestive failure had chief complaints of mental disorders, 4 had severe dizziness, 4 repeated syncope and cardiac pain was responsible for the admission of 3. In many cardiacs congestive failure commenced or became worse following strenuous exercise.

5. Three grades of severity of the anatomical lesions were established. There was a higher average admission rate for those with mild lesions (1.87) than those with moderate (1.36) or severe lesions (1.34), many of whom had only the one admission which terminated in death.

6. Group I patients had an increased frequency of admission in the fall months of September, October and November but no such trend occurred in Group II.

7. Group II patients were admitted for infection (endocarditis) and various diseases of old age. Injury following syncope was a minor though significant cause of admission.

8. Pulse rate was accelerated, but not to extreme degrees, averaging 96 for cardiacs, 86 for the others. Respirations were increased. Blood pressure was not characteristic—there were a number with systolic hypertension, others with low diastolic pressures but relatively few with the low systolic, low pulse pressure described as typical of aortic stenosis.

9. Enlargement of the heart was detected clinically in 78 percent of the cardiacs and 52 percent of the others. Muffling of the apical sounds was common. At the base the second sound over the aortic valve was usually absent or much reduced in intensity. Occasionally it was loud and must have been transmitted from the pulmonic valve. Comparison of second sounds in the right and left second interspaces was not always helpful since loss of A_2 or increase of P_2 were not regular in occurrence.

10. A systolic murmur was heard at the base in only 83 percent of the cases, with transmission into the neck vessels in slightly less than half. Basal diastolic murmurs were heard in a third of the cases. Apical systolic murmurs were heard in 82 percent of the cases and an apical diastolic murmur was noted in slightly less than a third. Thrills were felt in 33 cases. The systolic murmurs and thrills were related in intensity to the degree of stenosis but they were absent in several cases with severe valvular obstruction.

11. Manifestations of congestive heart failure were commonplace but were not of a peculiar variety except for an unusual frequency of sweating.

12. Fluoroscopic demonstration of calcified aortic valves verified the diagnosis 5 times. With routine study a much larger number would have been found.

13. Laboratory studies of blood and urine were not helpful in making a diagnosis.

14. Nothing pathognomonic was found in the electrocardiograms. Auricular fibrillation occurred in 19 percent of the cases, a higher incidence than reported elsewhere. Left axis deviation, conduction defects and signs of myocardial disease were frequent.

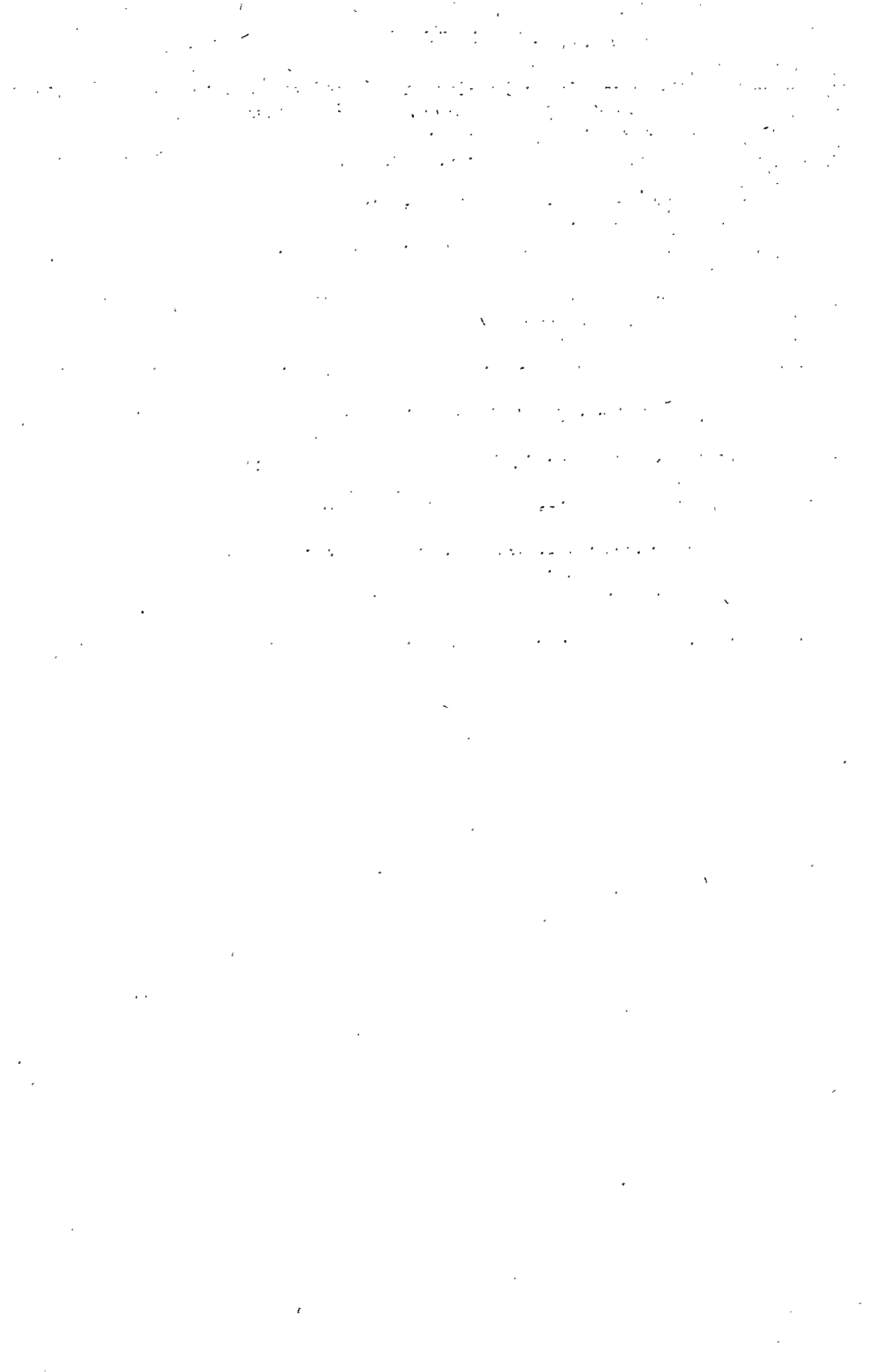
15. The hospital course was characterized by signs of congestive failure unusually refractory to treatment with digitalis, oxygen or diuretics. Episodes of sweating, cyanosis, restlessness and confusion occurred in 28 patients. They came and went uninfluenced by special therapy. Signs and symptoms referable to the brain were conspicuous in 13 patients.

16. Treatment was unsatisfactory. Digitalis and oxygen gave poorer response than is usually seen in other forms of congestive failure. Diuretics were only slightly more helpful.

17. Cardiac pain occurred in 37 percent of the patients before hospitalization and in 8 percent while under observation. It differed from typical angina pectoris in its lack of radiation or its radiation to the right, its advent after rather

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THE PATHOGENESIS OF SPLENOMEGALY IN HYPERTENSION OF THE PORTAL CIRCULATION; "CONGESTIVE SPLENOMEGALY"

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This study represents an extension of previous studies (1, 2, 3) on the sclerosis occurring in various organs subject to increased intraarterial or intravenous pressure.¹

THE HISTOLOGICAL ANATOMY OF THE NORMAL SPLEEN

Only the data pertinent to this thesis will be given. The red pulp is occupied by sinuses, between which is a labyrinthine structure consisting of narrow cords, the Billroth cords, the lacunae of which measure 6 to 16 μ . in diameter, and contain the free cellular elements of the blood. These lacunae constitute the main blood depot of the organ. The red pulp merges into the white pulp and the fibrillar reticulum of both are continuous. The Billroth cords are composed of a cytoplasmic and fibrillar reticulum. The cells are of the lymphoid type, larger monocytes having a pale round nucleus, with a cell body that varies in shape, which may be winged or sail-like or elongated. These have been termed "splenocytes", but they are not specific for the spleen. The cells of the pulp cords have received various genetic interpretations and nomenclatures, but no matter what terms are used it is necessary to stress, according to Klemperer (4) that this cytoplasmic structure may differentiate under morbid conditions along four different lines: 1) hematie, as evidenced in extramedullary blood formation; 2) phagocytic, either of free blood cells or pigment; 3) fibroblastic, with formation of newly formed fibrillar reticulum, histiocytes and collagen; 4) endothelial proliferation with formation of new sinuses. These potentialities are manifested in "congestive splenomegaly." The cytoplasmic pulp is viewed as a part of the reticulo-endothelial system. The cells of the pulp cords lie within the meshes of this fibrillar reticulum, and according to Mackenzie, Whipple and Wintersteiner (5) who viewed these fibrils in the living state by transillumination, the fibrillar reticulum supports the columns of pulp cells rather than envelops the intercellular channels. This fibrillar network of the red pulp is also continuous with that of the Malpighian bodies.

The relationship between reticulum and collagen has been much debated, but the view is now generally held that reticulum is a precollagenous substance (6, 7, 8, 9). Under any circumstance, the fibrillar reticulum under certain conditions, especially in congestive splenomegaly, may be transformed into collagen and the individual fibres may become hypertrophic and hyperplastic.

¹ I am deeply indebted to Dr. Rosen of Montefiore Hospital for one case, and to Dr. Jacob Furth of New York Hospital for three; the remainder came from the pathological laboratory of the Mt. Sinai Hospital.

THE VASCULAR SUPPLY OF THE SPLEEN

The splenic artery penetrates the capsule at the hilum and passes into the trabeculae with which it branches, the branches becoming progressively smaller. When they have attained a diameter of 0.2 mm. they leave the trabeculae and become sheathed by a cylindrical layer of lymphatic tissue. When the arteries reach a calibre of 40 to 60 μ . they leave the lymphatic tissue and enter the red pulp. Here they branch into small straight vessels, called the penicillary branches. These penicillary arteries have three successive parts: 1) the artery of the pulp, 2) the Schweigger-Seidel sheathed portion, and 3) the terminal capillaries of the pulp. The latter consist of endothelium supported by a few longitudinal fibres and elongated spindle shaped cells. These capillaries are surrounded by a network of reticulum continuous with that of the pulp. It is in the manner of termination of these pulp capillaries into the venous system that controversy has centered as to whether the spleen has an open or closed circulation. We need not enter at length into this controversy except to say that the weight of modern evidence is overwhelming in favor of an open circulation (10, 11, 12, 13, 14, 15, 16). The venous sinuses are from 12 to 40 μ . in diameter. Unlike the veins they are not lined by a flat vascular endothelium but by narrow cells parallel to the long axis of the vessel, with prominent nuclei that bulge into the lumen. The wall of the sinus represents only a flattened out cytoplasmic reticulum containing stomata. It is through these stomata that free blood cells lying within the meshes of the pulp cords of Billroth flow into the veins. The sinuses unite to form the pulp veins which enter the trabecular veins. These unite close to the hilus to form the splenic vein. To return to the terminal capillaries of the pulp, it has been amply demonstrated (16, 5, 12, 17) that these capillaries end in a funnel shaped dilatation, the ampulla of Thoma, which is also perforated like the splenic sinuses. This funnel enters directly into the meshes of the pulp reticulum. The interstices of the pulp therefore provide the only system of communication between the artery and the vein. These evidences of an open circulation derived from the observation and interpretation of histologic preparations have been convincingly confirmed in the living animal with the aid of quartz rod illumination technique by Mackenzie, Whipple and Wintersteiner (5). These observers also made a significant observation. They noted that the circulation in the pulp cords was greatly modified according to whether the organ was in a relaxed or in a contracted state. When it was contracted, as for instance after a hemorrhage, the pulp spaces collapsed so that the blood in these spaces short circuited almost directly from the arterial capillaries into the venous sinuses and the circulation appeared closed. This observation probably accounts for some of the erroneous interpretations in the past, and its significance in the interpretation of certain aspects of the finer morphology of congestive splenomegaly will be discussed later. According to Mackenzie and his coworkers, the pulp spaces in the relaxed spleen measure 6 μ . in width but the diameter of dilated ones is 16 μ . This potential dilatation together with that of the sinuses affords a measure of the distensibility of the normal organ, and accords with the observations of

McMichael (18) who found that the spleen could not be distended to more than three times its normal size

THE CAUSES OF HYPERTENSION OF THE PORTAL CIRCULATION

Hypertension of the portal circulation has many backgrounds in morbid anatomy and helps to integrate many physiological and morphological consequences that have hitherto been largely unclarified. Up to within a few years, hypertension of the portal circulation remained a concept, due to the work of Whipple and his coworkers (19) hypertension of the portal circulation is now a demonstrable fact.

A Cirrhosis of the liver The frequency of a much distended collateral circulation involving the esophageal veins is adequate evidence that a hypertension of the portal circulation occurs in hepatic cirrhosis, at least in advanced forms of the malady. The portal venous pressure was determined by Thompson (20) by simultaneous measurements in the splenic and antebrachial veins. The pressure in the splenic vein in cirrhosis varied between 225 and 470 mm. of normal saline solution while the pressure within the antebrachial veins was within the normal limits. According to Bellis (21) the normal portal pressure in man varies between 14 to 22 mm. of normal saline solution.

The determination of the presence or absence of portal cirrhosis by gross inspection of the organ is not always reliable, as McMichael (18) and Connor (22) have emphasized. Only a biopsy is conclusive.

Thompson (20) claims that biliary cirrhosis does not produce hypertension of the portal circulation. As will later be shown, changes identical to those found in portal cirrhosis were found in the spleen in nine cases. In one case of cirrhosis associated with hemochromatosis the same changes were noted, in two of hepatic lobatum and in five of toxic cirrhosis. Of Larrabee's (23) 47 cases of congestive splenomegaly, five occurred in hepatic lobatum.

In one case of schistosomiasis we found an extensive hepatic cirrhosis and changes in the spleen identical with those of portal cirrhosis. Thompson (20) believes that this parasite lends itself particularly to the production of portal hypertension. Such spleens are usually very large, weights of over 1000 gms. are not unusual. In our case it weighed 2250 gms. In six cases Thompson found invariably high pressures in the splenic vein in schistosomiasis, in one instance, it attained a height of over 500 mm. saline solution. It is evident that cirrhosis of any type or origin is capable of producing hypertension of the portal circulation.

B Chronic thrombosis of the portal or splenic veins This is the commonest extrahepatic cause of hypertension of the portal circulation. The clinical expression of the disease is dominated by hemorrhage into the gastrointestinal tract from rupture of the collateral esophageal veins. The spleens are as a rule larger, for the reason, we believe, that the portal hypertension is sustained over a more prolonged period than in hepatic cirrhosis. The clinical histories of such patients span, as a rule, a greater number of years. Portal cirrhosis kills earlier, because of potential hepatic insufficiency, which does not occur in prolonged

thrombosis of the portal or splenic veins. However, thromboses of the portal veins are by no means uncommon in portal cirrhosis. Under these circumstances, both the size of the spleen and the histological findings are intensified.

A primary phlebosclerosis, especially of the portal vein, has been frequently invoked as the cause of a thrombus, but the evidence is decidedly against such a contention, since a primary sclerosis of a vein probably never occurs. Available evidence shows (24), that a phlebosclerosis is always secondary to a prolonged venous hypertension. Fully organized portal and splenic vein thrombosis probably accounts for the majority of the cases of "Banti's syndrome" when at operation the liver is found smooth. According to Thompson (20), and we confirm his statement, these lesions never result in portal cirrhosis, thus nullifying the existence of Banti's "second stage." Rousselot (25) demonstrated a hypertension of the portal circuit in all cases of chronic splenic vein thrombosis.

The description of the evolution of the splenic changes from the acute and subacute thromboses represents, we believe, an important aspect of this study.

C. *Cavernous transformation of the portal or splenic vein.* This lesion should, strictly speaking, be included in the foregoing classification, since Klemperer (26) has brought conclusive evidence that it represents a terminal phase of canalization of a thrombus combined with the opening of new collateral channels within the lesser omentum. We shall describe the spleens in four cases of this lesion in the portal vein and three in the splenic vein. In the latter the lesion may be limited to this vein or represent an extension from the portal.

D. *Stenosis of the portal or splenic vein.* Of Larrabee's (23) 47 cases of congestive splenomegaly three were due to adhesions about the portal area following operation for appendicitis. We shall report four cases. In three, the vein was compressed by malignant neoplasms, in the fourth the cause was a distinct ridge at the junction of the splenic and the superior mesenteric veins. Whether this was congenital or not could not be decided; the splenic vein was greatly dilated. Billman and Pohl (27) have recently reviewed the subject of congenital stenosis of the portal vein.

E. *Thrombosis or endophlebitis of the hepatic veins.* On mechanical grounds one would surmise that obstruction of the flow of blood from the hepatic veins into the vena cava would give rise to a portal hypertension. Clinically this surmise is confirmed by the reports of an anastomotic circulation (28) and the frequent association of splenomegaly. Brandes (29) showed experimentally that mechanical constriction of the hepatic veins raised the portal pressure to twice its original level.

F. *The Cruveilhier-Baumgarten syndrome.* The hypertension of the portal circulation is consequent to the post-natal persistence of the umbilical vein. This malady is associated with a hypoplasia of the liver, a very large spleen and a collateral circulation. For a comprehensive and critical study of the malady, the reader is referred to the excellent paper of Armstrong and his associates (30). We have had no opportunity to study such a case.

G. *Hypertension of the portal circulation of cardiac origin.* It will be more appropriate to discuss this heading in a subsequent portion of this presentation.

THE MORPHOLOGICAL CHANGES IN THE SPLEEN IN HYPERTENSION OF
THE PORTAL CIRCULATION

Descriptions of the morphological changes in morbid spleens lose much of their value because the morphology has been viewed statically and not biologically. 86 cases have been studied and no case has been included in which the cause of the portal hypertension was not found. Most of the spleens were removed at autopsy; only very few were removed at operation.

Furthermore, the finer morphology of the liver has been studied in every case to note the relation of changes in this organ to those in the spleen. We have used the hematoxylin-eosin, the van Gieson, the Bielschowsky silver, and the Azan modification of Mallory's anilin blue fuchsin stains. Finally, the history of each case was studied to note the duration of the malady and whether certain clinical events, such as hemorrhage, infection, or an associated cardiac complication might modify the morphological picture.

a. *Portal cirrhosis*. 20 cases. Three were associated with primary carcinoma, two of the solitary type and one multicentric. The normal weight of the spleen, as of all parenchymatous organs varies within fairly wide limits depending upon the amount of blood that oozes out of the organ. Even the spleen associated with hepatic cirrhosis shrinks although it is firmer than the normal spleen. Spleens removed at operation weigh more than those removed at post mortem because the pedicle has been tied. Moreover, the size and weight of the spleen depend upon whether the patient has suffered from a recent hemorrhage or an associated infection, a valvular lesion or shock. Hence, the weight of the spleen removed at autopsy is by no means always a measure of the organ during life. According to Krumbhaar and Lippincott (31) the average weight of the normal spleen in adults is about 150 gms. This weight has been accepted as the standard.

The smallest spleen in this series weighed 230 gms., the largest 850 gms. The average was 458 gms. There was no correlation between the weight of the spleen and that of the liver, but there was a broad correlation between the weight of the spleen and the amount of fibrosis within the liver. By and large, the largest spleens occurred in cases where the amount of hepatic fibrosis overbalanced the epithelial content. However, there were exceptions.

The splenic capsule was thickened in 10 of the 18 cases. As a rule, this occurred in the largest spleens. The greatest capsular thickenings occurred in spleens in which subcapsular old pigmented hemorrhages or calcium incrustated hemorrhages were present.

Gross phlebosclerosis of the portal or splenic vein was found in two cases. The incidence would probably be higher had microscopic sections been made routinely. In the smaller veins of the trabeculae it was found in three, all in instances in which the main portal vein was studied and found free of sclerotic changes. It is also probable that the incidence of trabecular phlebosclerosis would have been higher had more sections of the spleen been made. In many of my cases only a single microscopic slide of the organ was available. Li (32)

found that in hepatic cirrhosis the incidence of portal or splenic vein phlebosclerosis was 77 per cent.

Widening of the Billroth's cords was the most consistent finding for it was absent in only one. In this case the spleen was comparatively small (350 gms.) and the sinuses were so compressed as to be barely visible. This spleen was imbedded in a mass of thick dense adhesions.

The Billroth cords were a mixture of early fibroblasts and fibrillar connective tissue in various proportions. A dominantly fibroblastic spleen was found in only one, and a complete terminal fibrous change was found in only one. This is in contrast, as we shall see, to prolonged portal or splenic vein obstruction in which a transformation to fibrillar connective tissue is predominant. The cells lining the sinuses have large conspicuous nuclei that bulge into the lumen; when the sinus is dilated the lining cells appear more flattened, like vascular endothelium. As a rule, sinuses are more readily visible in the subcapsular areas and adjacent to the trabeculae.

The erythrocyte content of the cords varied according to three main factors: 1) The maturity of the fibrosis; the more advanced fibrotic cords showed fewer. In completely fibrosed cords the amount was minimal so that the circulation was less open than normal. In the intermediary phases the red blood cells sometimes lie in narrow straight channels as though the cords had been capillarized. 2) An associated cardiac lesion. In four instances, the cords appeared more congested than one would expect from the degree of fibrosis. This interpretation was corroborated by an associated congestion in the marginal zone of the Malpighian follicles and dilatation of the sinusoids, conventional findings in congested spleens of central origin. 3) Collapse due to hemorrhage. In such instances the spleen contracts and the blood is squeezed out of the pulp spaces into the circulation, thus serving as an emergency measure (33). This is most conspicuous in the deeper areas where the trabeculae are smaller than in the subcapsular areas.

Increase in the trabeculae was noted in almost every case. This was associated in all by "trabekelaufsplitterung", a term devised by Dürer (34). By this he refers to the lack of the normally sharply defined borders of the trabeculae, so that the trabecular connective tissue merges into the surrounding pulp. This "auf-splitterung" varies in intensity and is generally proportionate to the maturity of the sclerosing process. It affects the smaller trabeculae earlier than the larger.

Simultaneously the fibrillar reticulum shows progressive thickening and hyperplasia. Hueck (15) uses the term "sclerosis" and Gauckler (35) the term "sclerose hypertrophische pulpaire" for this process. The fibrillar reticulum is the result of the fibroblastic potentialities of the cellular reticulum, and partakes in the increased formation of collagen. Macrophages showing siderophagocytosis and erythrophagocytosis were sufficiently numerous to be noted in nearly all. They were found both within and without the sinuses. Their presence or absence appears to have no essential significance. Plasma cells in small quantities were noted in three cases. Extramedullary blood formation was noted in only one case.

The increase in cellular and fibrillar reticulum rendered the sinuses clearly visible. The more the reticulum veered to the fibroblastic range, the more sharply defined the sinuses appeared to be, so that, when the fibrosis was nearly complete, the section assumed an angiomatous appearance. Sinus hyperplasia was not quite as conspicuous in the spleens of hepatic cirrhosis as in prolonged obstruction of the main portal vessels.

Sinus hyperplasia was present in all but one instance. This was a case in which the spleen was almost completely compressed. The sinuses were more numerous and conspicuous in spleens that showed more advanced fibrosis. The sinuses vary in shape, being more rounded and regular in fibrotic spleens than those in the early phases. They vary in width from those that are almost completely collapsed to those that appear dilated. These variations depend on a number of factors: 1) The stage of fibrosis. The more advanced the fibrosis, the larger the sinuses. 2) The state of contraction of the spleen. After a hemorrhage the sinuses appear more collapsed. 3) The size of the sinuses are particularly different in post mortem material where the blood is permitted to ooze freely than in spleens removed at operation where the vessels have been ligated. In the only case of cirrhosis of our series where splenectomy was performed the sinuses were conspicuously dilated. It is for this reason that many observers (34, 36, 37, 38) have stressed the need of examining the histology of the spleen in the distended state and after washing out the blood. 4) An associated cardiac lesion, in such instances the sinuses are wide.

The Malpighian bodies were almost invariably reduced in size. This was the result of fibrosis, either perivascular around the central artery or more frequently in the marginal zone. In a number of cases it was both. The degree and extent of this fibrosis varies and parallels that noted in the red pulp. The perifollicular fibrosis is not due to organization of a hemorrhage but to extension of the sclerosis from the red pulp. This is the lesion that Banti termed "fibroadenie" of the follicles and which he regarded as specific for the disease which he described. Since many observers have since shown that "fibroadenie," as Banti defined it, occurs in a wide variety of spleens that have been subject to prolonged portal hypertension, its specificity has been rejected.

Of 14 cases that came to autopsy and in which the presence or absence of a fairly developed collateral circulation, as manifested by varicosities of the esophageal veins, was noted in the protocol, eight showed such varicosities. The six in which it was not reported revealed large fatty livers in which the fibrosis was still in an early stage, with narrow zones of fibrosis, comparatively large liver lobules, and with little or no distortion of the vascular bed. This accords with the observation of Thompson (20) who observed that such patients were not subject to gastro intestinal hemorrhage but died usually from hepatic insufficiency. In two cases of portal cirrhosis fresh red thrombi in the portal vessels were found, the first in the left main branch of the portal vein and in the second there were mural thrombi of the splenic and superior mesenteric veins. Both showed midphases of the lesions. It is possible that the thrombi in the portal vessel may have enhanced the portal pressure.

That the stages we have described in portal cirrhosis are not hypothetical was proven in one case in which an accessory spleen the size of a pea was found. The morphological changes in the main organ are much more mature than in the accessory structure. The fibrosis is more mature, the Billroth cords are wider,



FIG. 1. PORTAL CIRRHOSIS WITH PRIMARY CARCINOMA LIVER
Spleen 725 gms. Low power. Marked sinus hyperplasia. Pulp cords fibrous

sinus hyperplasia is greater. The Malpighian follicles are smaller, and there is more "aufsplitterung" (figs. 1 and 2). We ascribe these differences to the much smaller venous tributary in the accessory organ in which the intravascular pressure must be less than in the main trunk.

b. *Biliary cirrhosis* 12 cases The causes for the cirrhosis were various Three occurred in infants with congenital obliteration of the bile ducts

Of the adult spleens, the smallest was 300 gms, the largest 1010 gms The average was 578 gms This was considerably higher than that found in portal



FIG 2 PFA SIZED ACCESSORY SPLEEN FROM SAME CASE AS FIG 1 SHOWING EARLIER LESION Sinus hyperplasia much less marked Pulp cords fibroblastic

cirrhosis, and indicates, as Klempeier (4) maintains, that icterus with perhaps an associated infection tends to enhance the size of the organ in biliary cirrhosis Phlebosclerosis of the portal vein occurred in three

Widening of the Billroth's cords occurred in all but one. The pulp cells reveal

the same progressive variations as in portal cirrhosis, but the general pattern was not quite as mature. The fibrillar and collagenous reticulum was considerably increased (fig. 3). In four instances, plasma cells were found in small numbers.

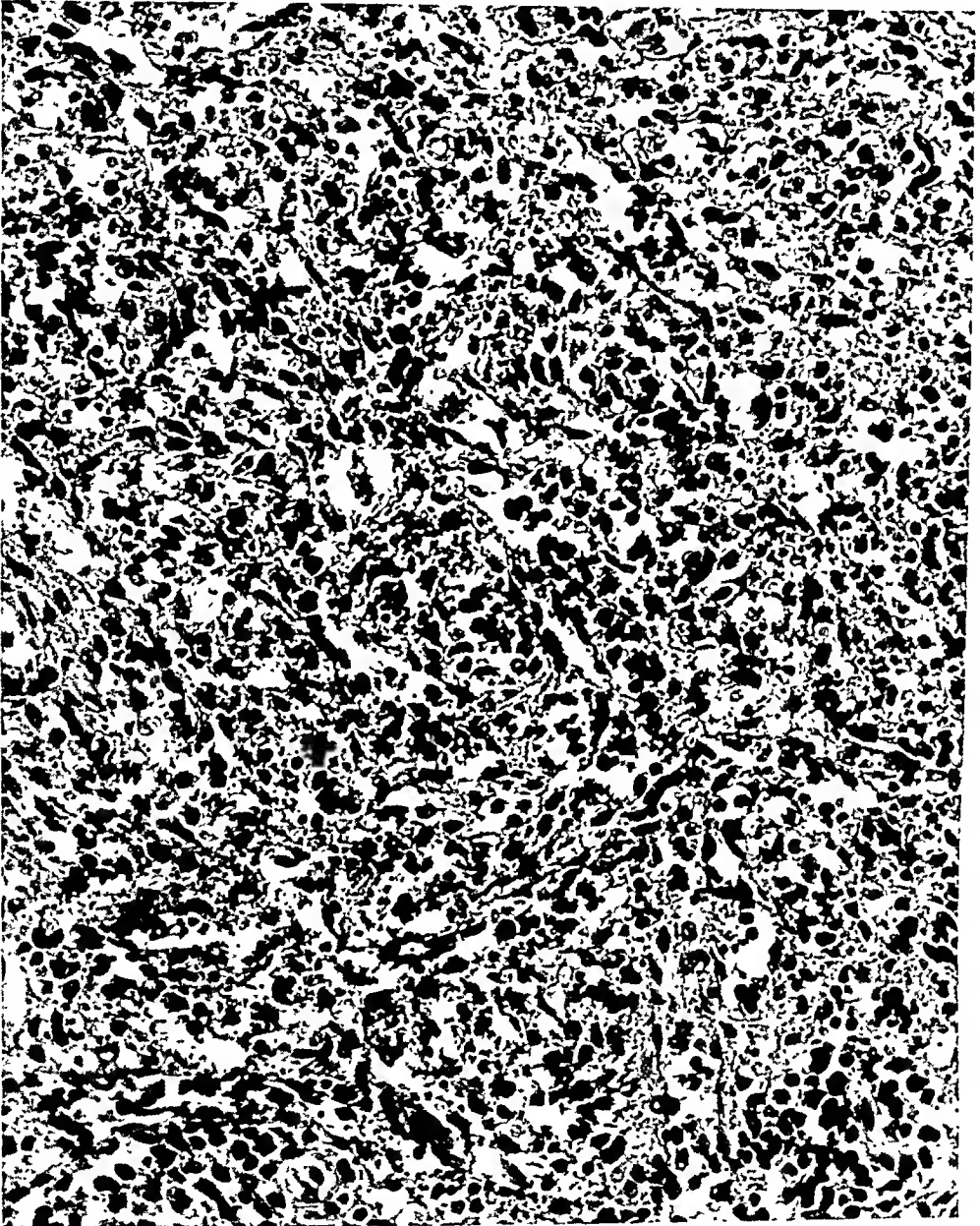


FIG. 3. BILIARY CIRRHOSIS DUE TO CHRONIC CHOLANGIOLITIS
Spleen 535 gms. Marked sinus hyperplasia, pulp cords wide and fibroblastic

Sinus hyperplasia was found in all but one, an infant of two months with congenital obliteration of the bile ducts. In the remaining two cases of congenital obliteration of the bile ducts, in infants of 6 and 7 months respectively, sinus hyperplasia was conspicuous.

Esophageal varices were found in three of the 12 cases, in two gastrointestinal hemorrhages occurred during life. The comparative infrequency of a well developed collateral anastomosis as compared to portal cirrhosis may be ascribed to the shorter duration of the disease. The factor of duration is illustrated in the three cases of biliary cirrhosis caused by congenital obliteration of the bile ducts. The infants died at the ages of 2, 6 and 7 months respectively. In all the morphological changes in the spleen are conspicuous, but less mature in the youngest than in the older two children. These cases in young children show that appreciable splenic fibroblastic changes can arise within a period of months.

c *Toxic cirrhosis* Five cases. The smallest spleen weighed 580 gms, the largest 800 gms. The average was 690 gms.

As in portal cirrhosis, gradations in maturity from the early fibroblastic to a fibrotic transformation of the pulp of the spleen was noted.

Only one of the four cases that came to autopsy showed esophageal varices.

Apparently, toxic cirrhosis through its more diffuse parenchymal involvement causes as a rule a wider distortion of the portal bed than portal cirrhosis, as the greater enlargement, the more conspicuous sinus hyperplasia and a greater degree of fibrosis of the spleens indicate. The comparative infrequency of the formation of a well developed collateral circulation may be accounted for, in part at least, by the shorter duration of the disease.

d *Hepatic cirrhosis with hemochromatosis* One case. The spleen was four times the normal in size and showed the characteristic changes we have described in advanced portal cirrhosis.

e *Hepatic cirrhosis due to schistosomiasis* One case. The spleen weighed 2250 gms. The finer morphological changes resembled those seen in advanced portal cirrhosis. There were areas of hematopoiesis. No phlebosclerosis was noted either in larger trunks or in the trabeculae. A collateral circulation was present with a ruptured esophageal varix. The unusually large spleen and the maturity of the lesions confirm Thompson's observations.

f *Hepar lobatum* Two cases. The spleens weighed 1240 gms and 650 gms respectively. Both spleens showed advanced fibrosis with sinus hyperplasia, thickened trabeculae, "aufsplitterung" and fibrosis of the Malpighian follicles. A few plasma cells were noted in the second case. Esophageal varices were present in both.

In respect to the maturity of the splenic morphology in these various types of hepatic fibrosis we would grade these spleens in the following order: 1) biliary cirrhosis, 2) portal cirrhosis, 3) toxic cirrhosis, hemochromatosis and hepar lobatum, 4) schistosomiasis. When the approximate date of onset of the obstruction can be determined, there is a correspondence between the maturity of the lesions and the duration of the obstruction. Whether there is any relation between the maturation of the lesions to the height of the portal hypertension could not be determined. Suggestive is the report of Thompson who found an average mean pressure of 350 mm. saline in seven cases of portal cirrhosis, while in six cases of schistosomiasis the average was 390 mm.

The extrahepatic causes of hypertension of the portal circulation are of special

significance in elucidating the pathogenesis, since they permit the study of the effects of the portal hypertension in the earliest stages.

g. Acute and subacute thrombosis of the portal vein. Five cases. These five cases represented various durations of the obstruction from a fresh red thrombus to those that were only beginning to organize, so that at the most the duration of the oldest clot may be safely put in terms of days or weeks. It is also necessary to add that in none was the obstruction complete. These five cases represent gradations of a process within a comparatively narrow range.

The earliest change was found in a case where the thrombus was fresh and red and almost completely filled the lumen. The spleen was already large, weighing 375 gms. The organ was a mass of red cells obliterating all the normal pulp landmarks. The sinuses were not visible and the lymphatic nodules were small due to the hemorrhagic infiltration. The pulp cells were widely scattered. The capsule was tense (fig. 4). The spleen cannot be distinguished from that observed in acute congestion from cardiac failure.

In another case where the thrombus was grey and firm, one noted the same congestion, but the sinuses were now visible but widely distended, especially in the subcapsular area. The fixed cells of the pulp are now more compact, but show no evidence of fibroblastic change (fig. 5). The marginal zone of the Malpighian follicles are still disrupted by erythrocytes. The spleen was shrunk somewhat.

The next stage is represented by a case in which the thrombus was grey and firmer. The sinuses were easily visible and distended. The endothelium is encircled by a delicate fibrillar network. Some of the cells begin to show fibroblastic changes. The congestion of the pulp cords between the sinuses is abundant, while the marginal zone of the lymph follicles is still hyperemic but less than in the preceding. The next stage is represented in an organizing thrombus of the left branch (fig. 6). The sinuses are increased and conspicuous, and are surrounded by a thin layer of collagen. The pulp cells are definitely fibroblastic. The pulp cords are wide and contain many red blood cells. The lymph follicles are small due to pericentric formation of collagen. The succeeding stage is represented in fig. 7. The sinuses are numerous and conspicuous. The pulp cells have been converted into fibroblasts. The Billroth's cords are wide and contain appreciably less erythrocytes than in the preceding four cases. There is as yet no or perhaps questionable "aufsplitterung". The Malpighian follicles are still small and show a narrow area of fibrosis in the marginal zone. A silver stain showed a moderate increase in the fibrous reticulum around the sinuses. In this case we found an accessory spleen which permitted us to compare the morphology in two different stages. The accessory spleen was pea-size and revealed only a diffuse hyperemia, such as was noted in the earliest stage. No sinuses were visible and the fixed pulp cells showed not the slightest evidence of a fibroblastic change (fig. 8).

The largest spleens in the group (375 gms. and 380 gms.) occurred in the earliest stages and approached the size (450 gms.) that McMichael (18) found to be the maximum state of distensibility of the normal human spleen. It is noteworthy

that the three spleens that showed the later stages weighed less, ranging from 175 to 245 gms. The livers in these cases showed no conspicuous changes.

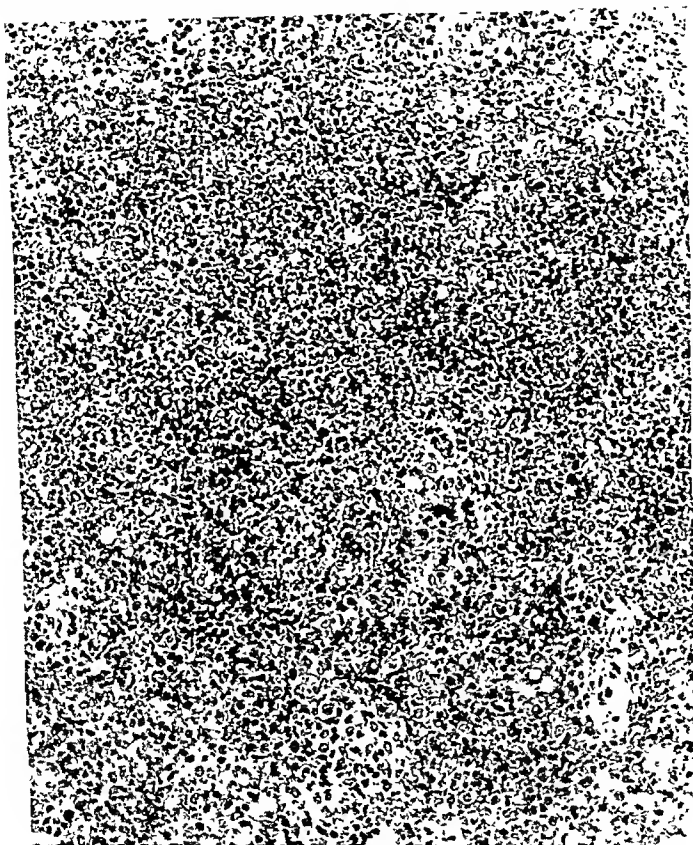


FIG. 4. EARLIEST PHASE OF PORTAL HYPERTENSION

Fresh red thrombus of portal vein. Spleen 375 gms., showing diffuse infiltration with erythrocytes, obliteration of sinuses, cells of red pulp dispersed (erythrocytes appear black).

The further progression of these initial changes will be discussed under the heading " cavernous transformation of the portal veins."

h. *Acute and subacute thrombosis of the splenic vein.* Four cases. These again revealed transitions. One showed a mixed red and grey thrombus, two showed

a grey thrombus, the character of the fourth thrombus was not described in the protocol. The largest spleen weighed only 225 gms. The smallest 210 gms.

The changes in this series show a slightly advanced stage as compared to

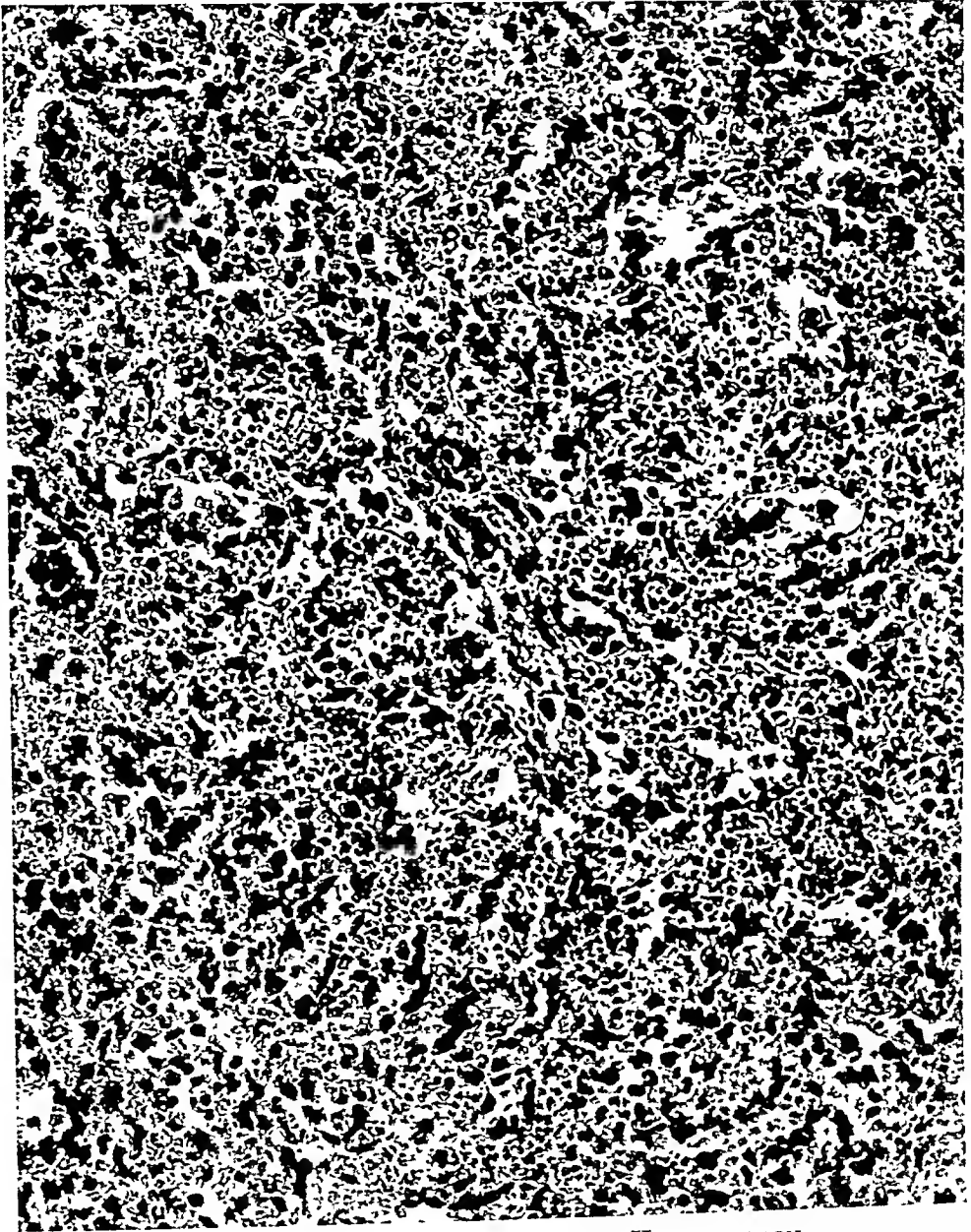


FIG. 5. SECOND PHASE OF PORTAL HYPERTENSION

Grey thrombus with partial organization of portal vein. Spleen 185 gms. Sinuses are now distinct; pulp cords between sinuses filled with blood.

thrombosis of the portal vein, but this may be due to the more advanced stage of the thrombi. The number of cases is too small to permit the conclusion that in acute or subacute splenic vein thrombosis the lesions are more mature than in acute or subacute thrombosis of the portal vein. The ultimate stage of splenic

vein thrombosis will be discussed under the heading "cavernous transformation of the splenic veins."

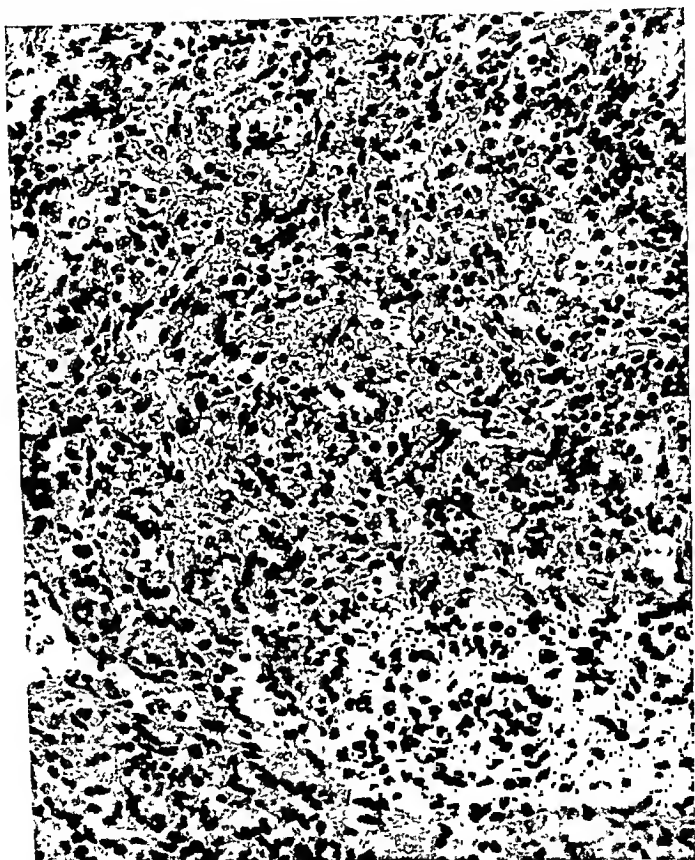


FIG 6 THIRD PHASE OF PORTAL HYPERTENSION

Old organized thrombus of portal vein following carcinoma of bile duct. Spleen 175 gms Sinus hyperplasia, pulp cords wide and fibroblastic.

Fig. 1. *Thrombosis of both portal and splenic veins.* Two cases. In the first case, with a fairly fresh thrombus of the splenic vein and a grey thrombus in the portal, the spleen weighed only 150 gms. The spleen showed the earliest lesion described previously.

The second case was of unusual interest because the diagnosis of "Banti's

disease" was made and a splenectomy was performed. The splenic and portal veins were filled with a firm carcinomatous thrombus. Esophageal varices were found, one of which had ruptured. The spleen weighed 1000 gms. The

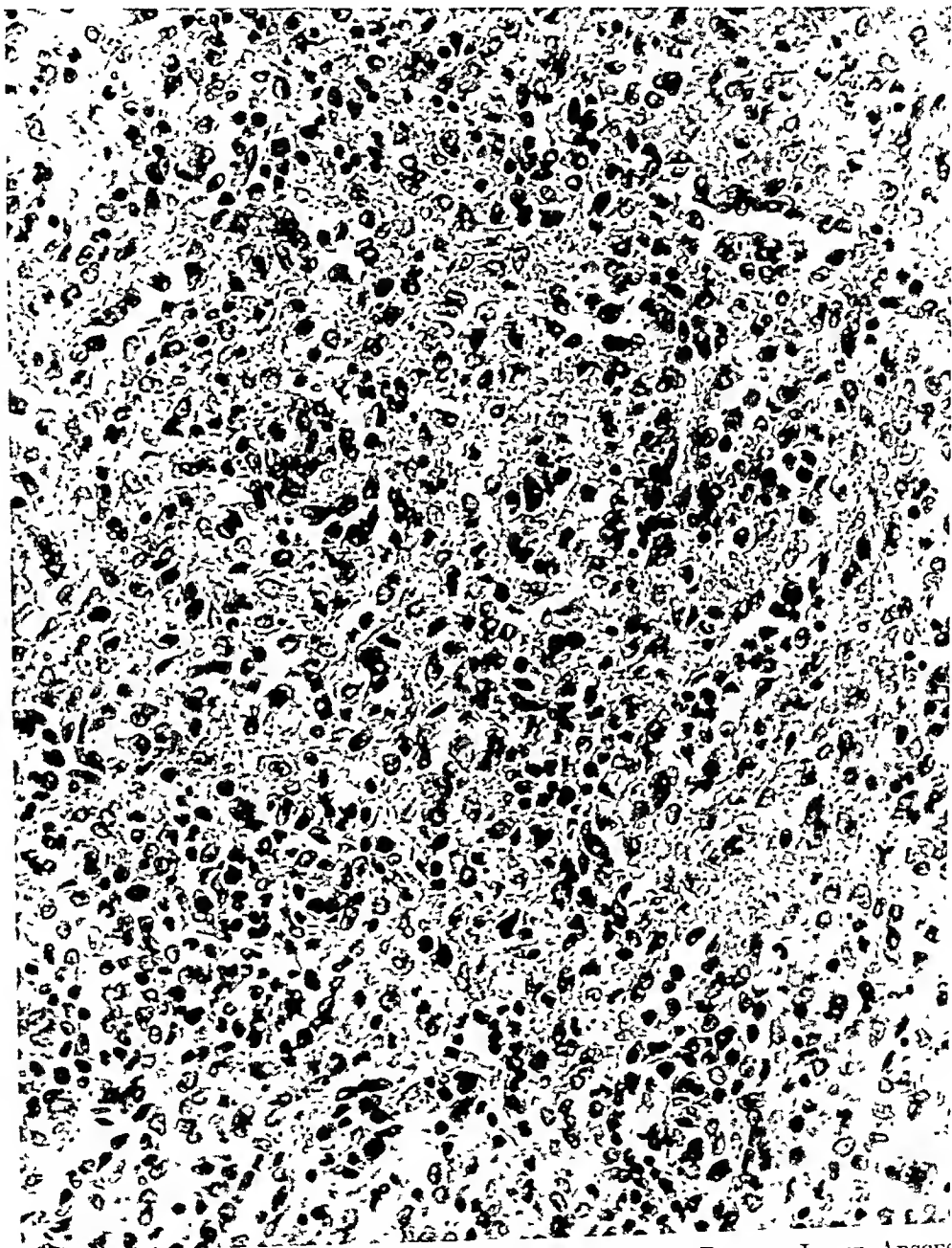


FIG. 7. SUPPURATIVE THROMBOPHLEBITIS OF PORTAL VEIN DUE TO LIVER ABSCESS
Spleen 245 gms. Sinus hyperplasia, pulp cords fibroblastic

histological picture is that of the fairly advanced stage of congestive splenomegaly. This case is significant because in view of the malignant nature of the tumor, the duration of the occlusion must be measured in terms of months; never-

theless the spleen was unusually large and the clinical picture was typical of "Banti's disease".

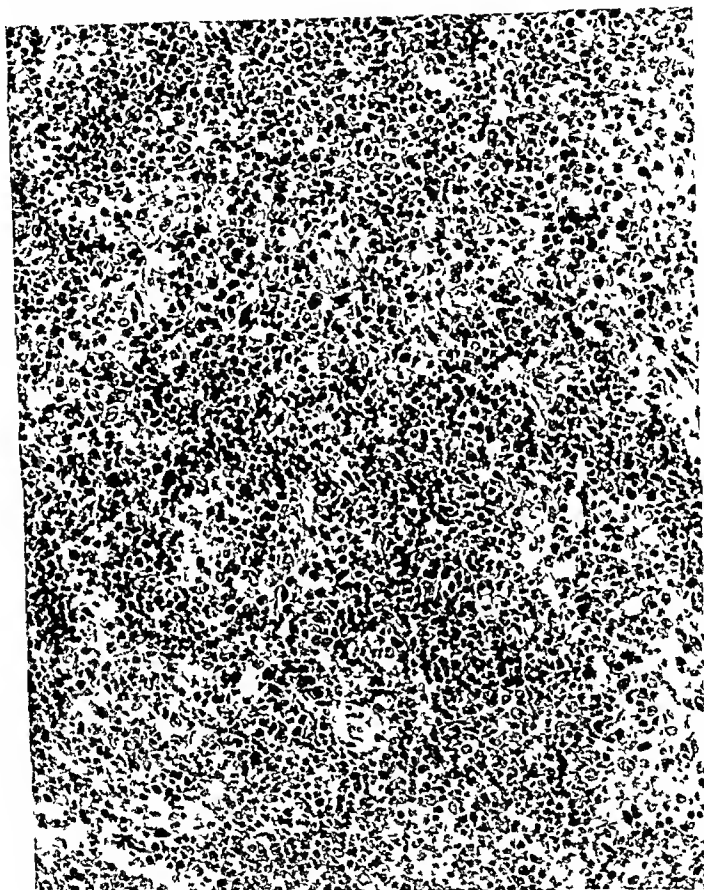


FIG 8 PEA SIZED ACCESSORY SPLEEN FROM SAME CASE AS FIG 7, SHOWING A MUCH FARTHER LESION (RED BLOOD CELLS APPEAR BLACK)

1) *Cavernous transformation of the portal vein* Four cases The weight of the spleens ranged between 700 and 1200 gms The characteristic morphology is represented in fig 9 The changes represent advanced fibrotic transformation of the pulp, marked sinus hyperplasia and thickening of the trabeculae, "auf-

splitterung", and fibrosis of the Malpighian follicles. Phleboscclerosis of the splenic vein was noted in 3 of the 4 cases. These showed varicosities of the esophagus. In three, typical megakaryocytes and foci of hematopoiesis were

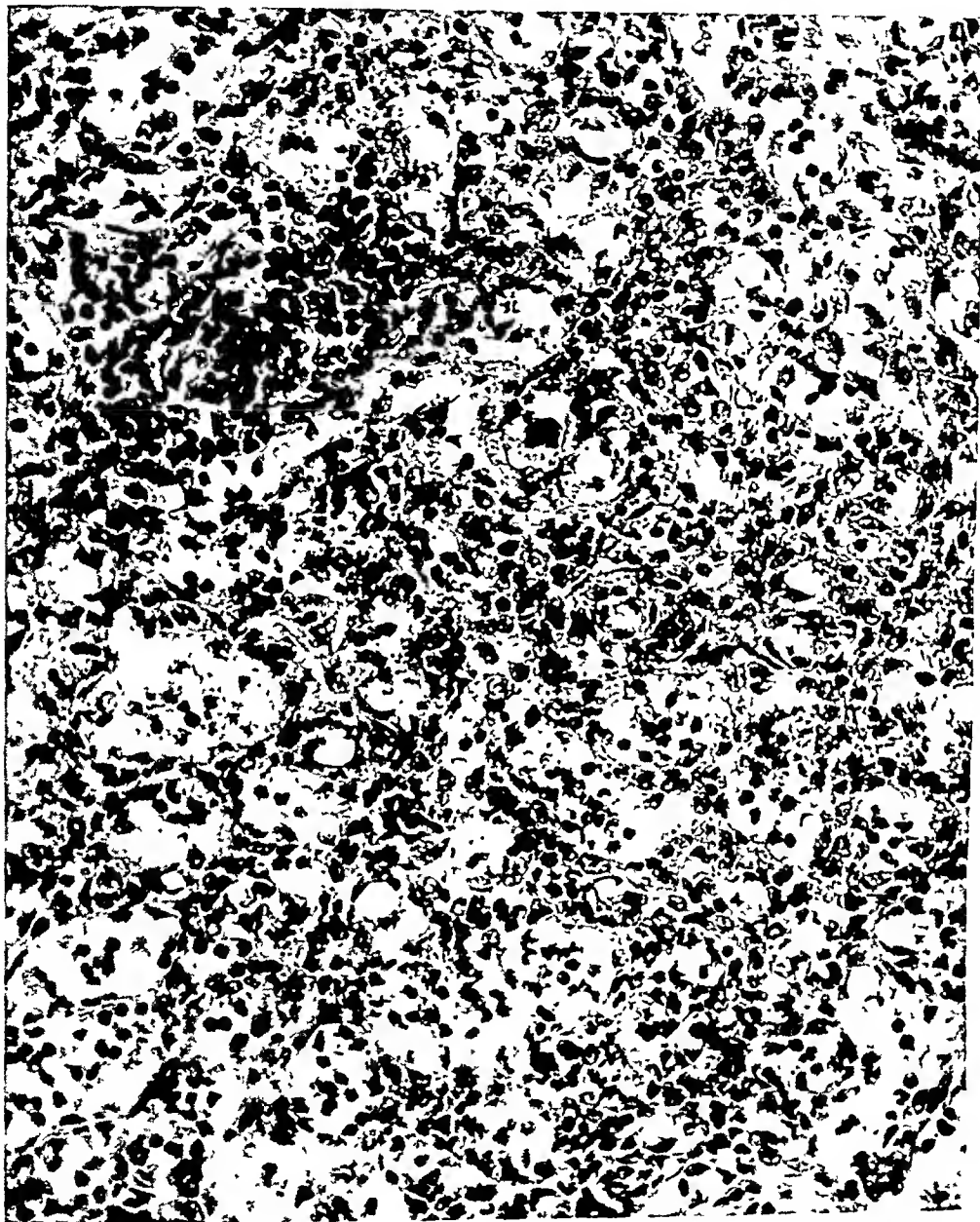


FIG. 9. LATE PHASE OF PORTAL HYPERTENSION

Cavernous transformation of portal vein. Spleen 6 × 11 × 21 cm. Sinus hyperplasia, pulp cords wide and fibroblastic.

seen. The livers appeared normal except for extension of the cavernous process into some of the portal radicles. These four cases revealed the classical morphological features of the spleen that have been described under the heading of "Banti's disease," "Splenic anemia," "Splenic fibrosis," "Sclerose pulpaire," and

"Congestive splenomegaly." It is obvious, in reviewing our previous exposition, that these changes are not specific for cavernous transformation of the portal



FIG. 10. TERMINAL PHASE OF PORTAL HYPERTENSION

Cavernous transformation of splenic vein. Spleen $24 \times 15 \times 6$ cm. Low power. Extensive sinus hyperplasia, mature fibrosis. Trabeculum shows "aufsplitterung."

vein but represent an exaggeration and a maturation of the lesions previously described.

k. *Cavernous transformation of the splenic vein.* Three cases. The first patient had an echinococcus cyst of the liver removed eight years previously. The spleen weighed 425 gms. In the second patient the spleen weighed 1000 gms. The

third patient presented a "Banti syndrome" with repeated hemorrhages and had a splenectomy. The spleen measured 24x15x6 cm.

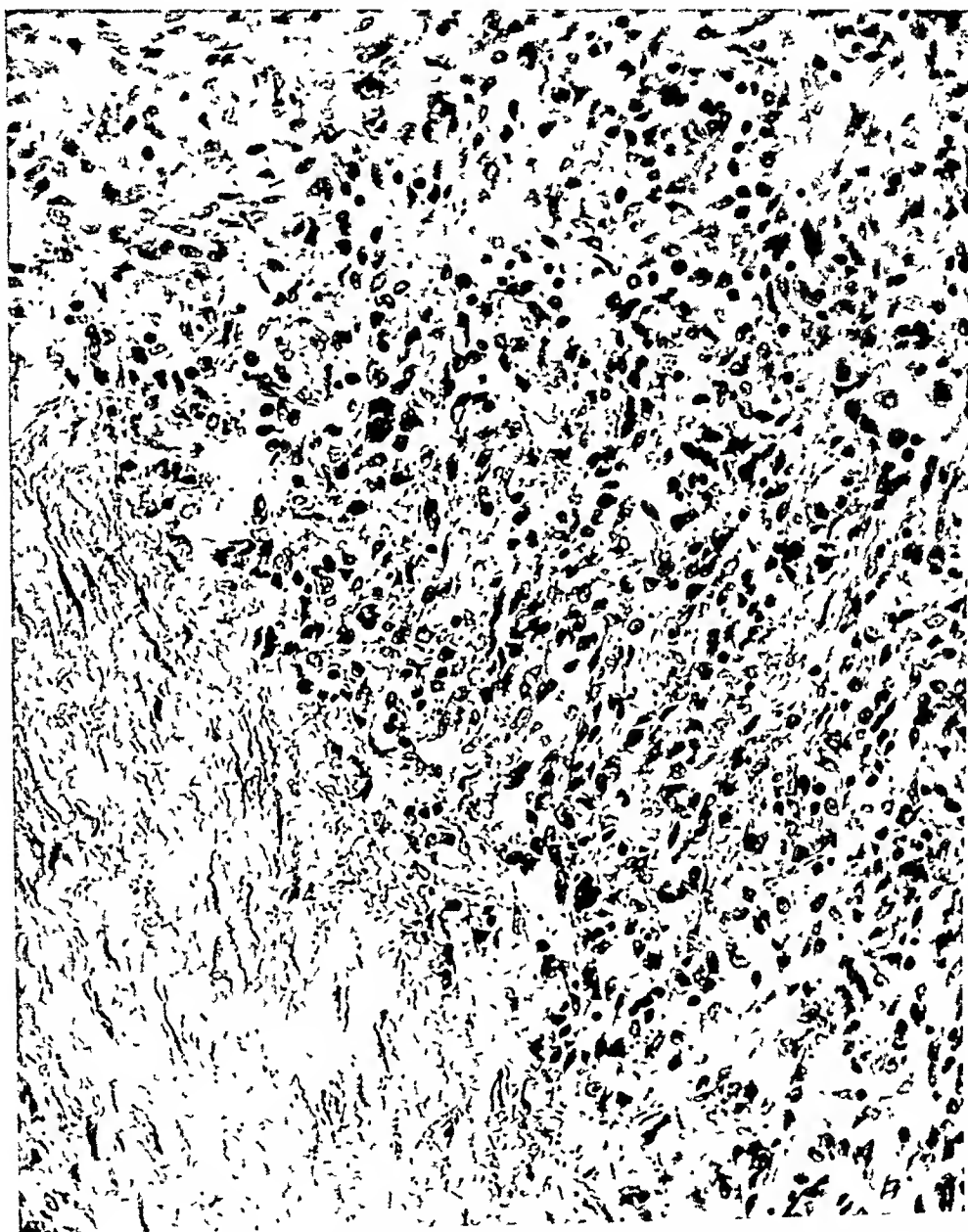


FIG 11 HEALED THROMBOSIS OF HEPATIC VEINS

Spleen 475 gms. Low power. Sinus hyperplasia with more mature fibrosis of pulp cords. Trabeculum shows "aufsplitterung".

These cases of old splenic vein thrombosis differed little from those following cavernous transformation of the portal veins. In one case the Billroth cords were almost completely fibrotic. In other words, the circulation of the pulp had been practically destroyed and the circulation within the organ had been converted from an open into an almost closed one. The section presented an

angiomatous appearance (fig 10). This case showed the most advanced lesion encountered and may be regarded as the ultimate stage of the process described

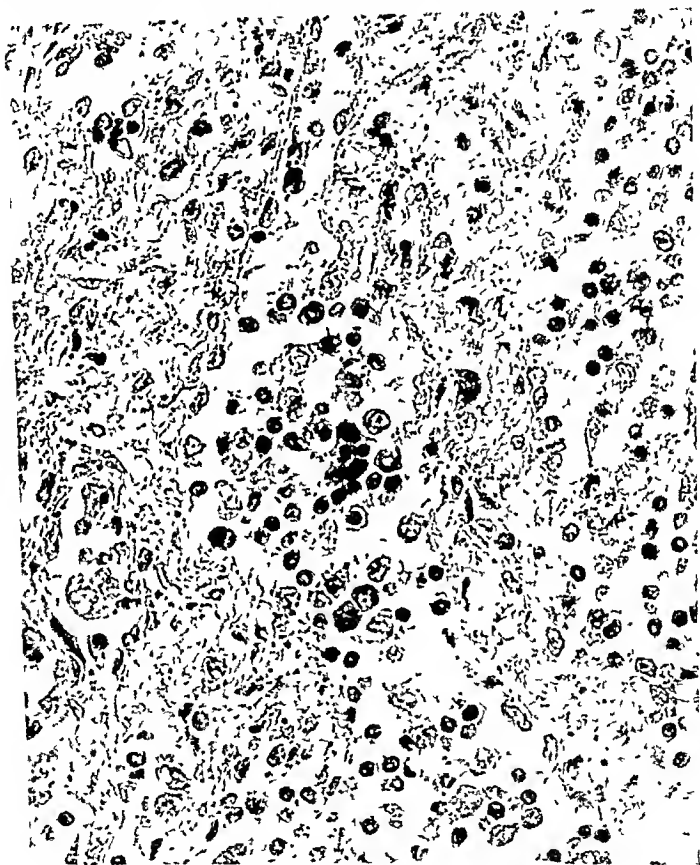


FIG 12 HEAVY THROMBOSIS OF HEPATIC VEINS
Spleen 475 gms Area of extramedullary hematopoiesis within sinus High power

This case supports the observation of Rousselot (25) that the largest spleens in hypertension of the portal circulation occur in those in which the obstruction is nearest the splenic hilus. In all three cases there were some mononuclear eosinophiles, many megakaryocytes and foci of hematopoiesis within the sinuses. Siderotic hemolysates were noted in some trabeculae and in the subcapsular

zone. The Malpighian follicles were extremely small and showed marked sclerosis of the marginal zone; and in one instance, there was central sclerosis. The two patients that came to autopsy showed esophageal varices.



FIG. 13. PHLEBOSCLEROSIS OF HEPATIC VEINS
Spleen 475 gms. Silver stain. Hyperplasia of reticulum around sinuses

1. *Compression and obliteration of the splenic veins.* Four cases. The compression was due to a neoplasm in three cases. In the fourth, the splenic vein was obliterated due to an old pancreatitis.

These cases reveal progression of the lesions from the earliest phase which we

have observed in acute and subacute thrombosis of the portal or splenic veins, to the late phase which we have described among the chronic obstructions. Presumably, the dominating factor in producing these progressive stages was the duration of the obstruction. In the earliest, the duration must have been extremely short, in the terminal, the duration must have been many years.

m Stenosis of the portal vein. One case. The spleen was three times the normal size. The capsule was thickened, the Billroth cords were widened and the cells were young fibroblasts. The sinuses were moderately hyperplastic. The trabeculae showed no "aufsplitterung." The Malpighian bodies were small. There was some perfollicular congestion.

n Obstruction of the hepatic veins. Two cases. While a splenomegaly of considerable size is recorded in all reports of obstruction of the hepatic veins, no study of the finer morphology of the spleen has been available, except that of Coronini and Obelson (39). They report a "fibroadenoma" analogous to that of "Banti's disease" in one case of endophlebitis of the hepatic veins. We were surprised to find such advanced lesions in our two cases.

The first was a male aged 19, who showed at post mortem old adherent and partially organized thrombosis of two of the main branches of the hepatic vein. There was marked fibrosis and sinus hyperplasia, the sinuses were wide and in conjunction with the fibrotic walls gave the section an angiomatous appearance (fig 11). A number of megakaryocytes were noted within the sinuses and areas of erythropoiesis (fig 12). The second case was one of endophlebitis of the hepatic veins, with complete occlusion of the lumina by dense fibrous tissue. The spleen was three times the normal size and showed fairly advanced hyperplastic changes (fig 13). The first case showed a somewhat more advanced stage than the second, but in both the degree of fibrosis is considerable and the lesions are indistinguishable from those we have described in the hepatic cirrhoses. No evidence of an anastomotic circulation was found in our case, but Thompson and Turnbull (28) report esophageal varices in two cases.

We have not had an opportunity to examine the spleen in the Cruveilhier-Baumgarten syndrome but the circulatory disturbance indicates strongly that a hypertension of the portal circulation must arise. A considerable splenomegaly is the rule in this disorder. Benque (40) and Eppinger (41) describe a morphology in the spleen comparable to that of "Banti's disease."

DISCUSSION

The material assembled thus far reveals a series of spleens with a consistent morphological biological progression, from a pure hyperemic spleen to one with such an extensive fibrosis that almost complete destruction of the pulp spaces has taken place, so that for the most part the terminal arterioles communicate directly with the sinuses. The early phases arise only when the obstruction in the portal circuit is of short duration, days or weeks, while the ultimate phases are always associated with an obstruction of many years duration. The common denominator is a hypertension of the portal circulation. To the older stages, the term "congestive splenomegaly" at Lariabee's suggestion, is conventionally

employed. Most students of the splenomegaly of "Banti's disease" or of hepatic cirrhosis have repeatedly postulated that congestion alone could not be held responsible for the changes, since spleens with this morphology are not observed in congestion of central origin. They have invoked another factor, perhaps a toxic or an inflammatory one. Furthermore, the proponents of a secondary factor did not take into consideration the fact that central congestion is not all of the same type, in terms of pathological physiology; they have curiously fallen into a common fallacy in assuming that "congestion" and "stasis" are synonymous with increased venous pressure. The difference becomes obvious in the clinical study of cardiac failure. The venous pressure only becomes elevated during periods of failure. When compensation ensues the venous pressure returns to normal levels, although clinical and even anatomical evidence of congestion remain. Congestion may be the result of factors other than increased venous pressure. In uncomplicated cases of polycythemia vera the systemic congestion is the result of an increase in blood volume; in hyperthyroidism it is due to an increased blood flow. We believe that the reason why the morphological changes in congestive splenomegaly are usually not observed in cardiac disease is because in most varieties of cardiac disease, the venous hypertension is a transitory one and not sufficiently prolonged. For this reason, we first studied the morphology of the spleen in a cardiac disorder in which increased venous pressure may persist for years, namely constrictive pericardium. Despite the clinical awareness that the spleen is enlarged in constrictive pericardium (42, 43) the finer morphology has not been studied.

o. *Constrictive pericardium*. Ten cases. In all but one of the cases we shall report, the rigidity of the greatly thickened pericardium was augmented by either circumferential or scattered areas of calcification. All showed the classical clinical evidence of this malady. In five in which readings of the antebrachial pressure was recorded, the pressures ranged between 150 and 285 mm. of normal saline solution. In three cases the hepatic veins showed thickening and areas of sclerosis, evidences, as we tried to point out some years ago (3), of prolonged elevated venous pressure. In one the splenic vein also was thickened. The spleens were all enlarged except three. The smallest weighed 44 gms. the largest 570 gms. The average was about 300 gms. The capsule was thickened in all; but in two cases it was sufficient to be termed "sugar casing." The Billroth cords were widened and showed fibroblastic to fibrotic transformation. The sinuses were hyperplastic, mostly with wide lumina, some with flattened endothelium, but they were not as prominent or numerous as in the cases of chronic obstruction of the portal or splenic veins or even in hepatic cirrhosis. The trabeculae were usually increased and "aufsplitterung" was noted in three that showed a preponderant fibroblastic change. The Malpighian follicles were usually small and showed some perifollicular fibrosis and in one case this was accompanied by fibrosis around the central vein. A typical spleen in constrictive pericarditis is shown in fig. 14. In comparison to the cases of chronic obstruction of the portal or splenic veins and to cirrhosis, the number of erythrocytes in the pulp meshes and the enlargement of the sinuses were greater than would be

expected from the degree of fibrosis. There were no trabecular hemorrhages, blood "seas" within the pulp or subcapsular hemorrhages, fresh or old. The livers were mostly enlarged, and in six cases showed a cardiac fibrosis. That

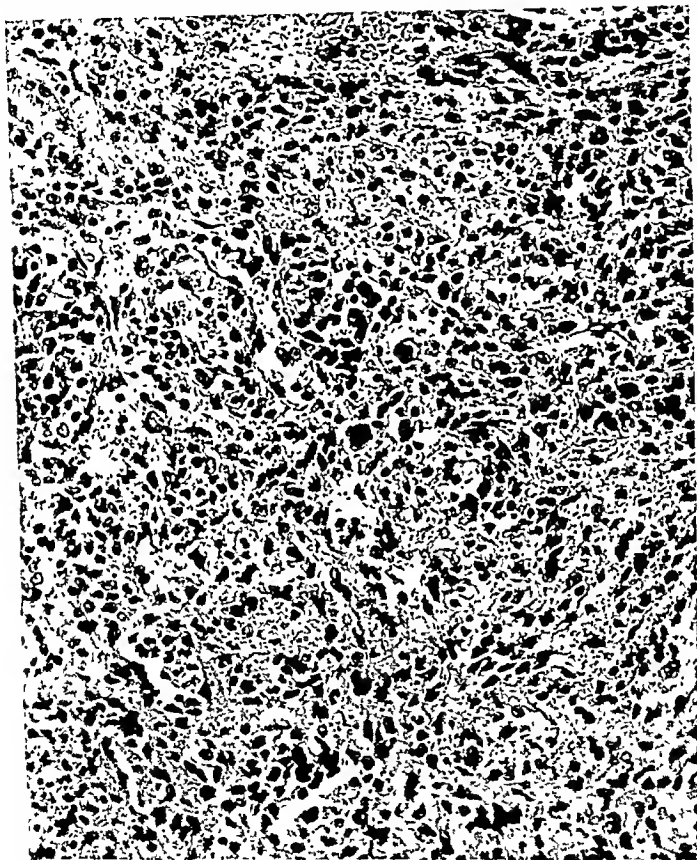


FIG. 14. CONSTRUCTIVE PERICARDITIS

Spleen 235 gms Sinus hyperplasia, widening of pulp cords which are fibroblastic. Two macrophages within sinuses are visible in centre of field.

this type of fibrosis is not essential to produce the lesions in the spleen is shown in one case in which fairly advanced lesions were found although the liver was not fibrotic.

In no case had any collateral circulation formed, nor indeed, would any be

expected. Collateral circulation arises only when an area which has been subjected to a prolonged high venous pressure must divert its blood into an area where normal venous pressures prevail. In constrictive pericardium, both portal and peripheral venous pressure are presumably equally elevated.

It seemed therefore pertinent to study the spleen in certain types of valvular defects, in which recurrent frequent attacks of decompensation occur with corresponding prolonged periods of elevation of venous pressure. Such cases arise either in advanced mitral stenosis with relative tricuspid insufficiency or cases of organic tricuspid insufficiency or stenosis.

p. *Cardiac hepatic fibrosis*. Ten cases. In all the right auricle and ventricle were enormously enlarged. The pulmonary artery was arteriosclerotic, and there was marked sclerosis of the capillaries of the pulmonary alveoli, expressive of an unusually severe and prolonged hypertension of the pulmonary circuit (2). As an additional evidence of marked hypertension of the pulmonic circuit, sclerosis of the hepatic vein was noted in five. The venous pressure was recorded in two cases; it measured 205 and 310 mm. of saline solution. In none was sclerosis of the portal or splenic veins found, but in one there was slight sclerosis of a trabecular vein. Li (32), on the other hand, reported phlebosclerosis of the portal vein in four cases of myocardial failure. The smallest spleen weighed 135 gms., the largest 360 gms., and the average was 245 gms.

Histologically, these spleens revealed progressive changes from those that were almost purely hyperemic to those that showed a greater or lesser degree of fibrosis with sinus hyperplasia. Even the most mature lesions had not passed the fibroblastic phase. There is always some perifollicular congestion. Silver stains for fibrillar reticulum show only a slight increase. The trabeculae in the later stages were increased; and only in the more fibroblastic spleens was there evidence of a beginning "aufsplitterung." The livers in these cases were usually large, and showed a greater or lesser degree of fibrosis around the central veins.

In summary, the changes noted in these spleens represented a less mature process than those observed in the cirrhoses of the liver and in chronic portal or splenic venous obstruction, but both in the evolution and in their morphology, these spleens were indistinguishable from those we have described in the subacute phases of extrahepatic obstruction.

q. *Cardiacs without cirrhosis*. Five cases. The cardiac lesions paralleled those of the preceding group. The weight of the spleens varied between 125 (a boy of 15) and 300 gms. The average was 205 gms. Histologically, the organs differed from the preceding only in the fact that all but two represented the early stages described previously. These latter showed lesions that were comparable to the later phases. The livers were large and showed extensive central congestion. It is apparent that the presence or absence of fibrosis in the liver has a slight influence in producing fibroblastic change within the spleen, and it does so only by virtue of the fact that the fibrosis represents a more prolonged phase of hypertension of the lesser circulation (3).

There is a paucity of studies on the finer morphology of the spleen in long valvular disease with passive congestion. Sokoloff (44) found

dilated sinuses with thickening of the adventitial walls and of the supporting structure of the pulp, which extends into the reticulum of the Malpighian bodies. The traheculae were thickened. Nieolaides (45) in addition found thickening of the trahecular veins. Matsui (46) describes hyperplasia and hypertrophy of the reticulum fibres, both of the pulp sinuses and of the Malpighian follicles. The greater the amount of reticulum the less cellular the pulp, and the larger the spleen the more advanced are the lesions. Stengel and Fox (47) found large spleens, less distinct Malpighian bodies, and hyperplastic traheculae and fibrous stroma. Klemperer (4) states "that in prolonged cases of passive congestion, the Malpighian bodies may decrease in size. The cytoplasmic reticulum and the free white cells of the pulp may diminish in number. The reticulum fibers become slightly prominent and the capsule and traheculae thickened." Smith and Gault (48) state that the ultimate phase of chronic passive congestion may render it difficult to differentiate the lesion from "Banti's disease" or hepatic cirrhosis. Whether all such spleens result in cyanotic atrophy, it is impossible to say. Hypothetically such an eventuality would be unlikely if a prolonged venous pressure is not maintained, as in constrictive pericardium.

COMMENT

From this exposition it is evident that the peripheral resistance may be anywhere between the heart and the hilus of the spleen. On mechanistic grounds one would presume that, *other things being equal*, the more distal the peripheral resistance is from the spleen, the less the portal hypertension. Consequently morphological changes would attain a lesser degree of maturation. Such indeed has been found to be the case. But there are influences that may modify the morphological picture profoundly. The most important is the duration of the obstruction. This is manifest in the marked difference in the morphology of the spleen in acute, subacute and chronic vascular obstructions. Another factor is unquestionably the degree of hypertension within the portal circulation. That the height of the pressure is a factor is indicated by Thompson's (20) observation that the greater the density and distortion of the fibrosis in portal cirrhosis, the greater the splenomegaly. The height of the pressure is not as important as the duration since the portal venous pressures do not approach the normal arterial pressures. Nevertheless, even under such pressures veins do become sclerotic (24), but only under conditions where the pressure has been prolonged such as in varicosities of the lower extremities, in the venous segment of an arteriovenous aneurism, and in the pulmonary veins in hypertension of the pulmonary circulation. Another influence that may modify the finer morphology is the presence or absence of cardiac failure. In this situation the sinuses, the pulp and the perifollicular areas are engorged. An additional factor is collapse of the organ consequent upon exsanguination. This is especially notable in the deeper portions of the spleen. Splenic collapse after hemorrhage has been emphasized by Lubarsch (49) and Brandberg (50). Brandberg has observed that fibrotic spleens contract less. Finally, an associated infection, with infiltration of the spleen with plasma cells may alter the morphology somewhat. We have noted such

when the thrombus has become grey and adherent, the sinuses again become visible but are widely distended and the lining endothelium is flattened. The Billroth cords are narrow and the pulp spaces are distended with erythrocytes. The cells of the pulp are not as dispersed and remain normal in appearance. The periphery of the Malpighian bodies are hyperemic but the zone is narrower. The spleen has shrunk somewhat. In the next stage, when the thrombus has become partially organized, the Billroth cords begin to widen; the pulp cells increase and have undergone a fibroblastic change, and the pulp spaces show an appreciable diminution in erythrocyte content. Well defined sinuses appear which are still dilated and the lining endothelium is no longer flat. The walls of the sinuses are composed of a delicate ring of fibrillar reticulum surrounding the endothelium. The Malpighian bodies are small and the hyperemic peripheral zone is now minimal. The spleen is still larger than normal but not appreciably more than the preceding. When the thrombus has become firm and well organized, the Billroth cords become still wider and the pulp spaces contain less erythrocytes. There are no longer normal pulp cells; they are much increased and many have been converted into fibroblasts. The sinuses are abundant, less dilated and the ring of the fibrillar reticulum around the sinuses is thicker and denser. The Malpighian bodies no longer show a hyperemic zone. In this stage the capsule may show some thickening and the spleen is larger. When the thrombus has become completely fibrous the Billroth cords are wide and the pulp spaces contain only a minimal quantity of blood. The pulp cells become predominantly fibroblasts and the sinuses show extensive hyperplasia and are even more prominent, so that the section begins to appear angiomatous. The walls of the sinuses are thicker. The trabeculae are increased in size and the edges begin to show an "aufsplitterung." The Malpighian bodies are smaller due to peripheral encroachment by the fibroblastic tissue of the pulp. The spleen is now much larger than normal and the capsule is more frequently thickened. A collateral circulation around the lower end of the esophagus is frequently present. When the thrombus becomes so completely organized as to be termed "cavernous transformation," the maturation of the process is almost complete. The Billroth cords become sclerosed and canalized and the pulp spaces contain only a small number of erythrocytes. The pulp cells are now all flat fibroblasts; the sinuses are sharply defined and the lining endothelium is unusually prominent. Their number is excessive. The Malpighian bodies are much smaller and in addition to a perifollicular, a fibrosis around the central vessel may appear. The trabeculae show distinct increase and there is a marked "aufsplitterung." Hemorrhagic areas within the trabeculae or in the subcapsular areas may occur, which are often siderotic or infiltrated with calcium. (McMichael (18) by serial section has shown that these arise from rupture of trabecular vessels.) Phlebosclerosis of the main trunks of the portal veins or within the trabeculae is frequent. Megakaryocytes may appear in fair abundance within the sinuses with areas of extramedullary hematopoiesis. The spleen is huge, averaging around 1000 gms. and the capsule is usually thickened. The greatest thickenings are in the immediate vicinity of the subcapsular siderotic hemorrhages. In all such cases a collateral

circulation has been well established. The maximum phase was noted in one spleen, the result of cavernous transformation of the splenic vein. Here the Billroth cords were almost completely transformed into firm fibrous connective tissue so that in many areas they appeared bloodless. The other splenic components were correspondingly affected. In this organ, the splenic circulation had been converted from an open to a practically closed one. All stages except the terminal, were noted in spleens that resulted from the other causes of hypertension of the portal circulation that we have enumerated. The reason the lesions have not progressed further is because the clinical histories and the nature of the obstruction leaves no room to doubt that the hypertension has not been sufficiently prolonged.

The general picture may be modified by certain secondary factors. With an associated cardiac lesion, perfollicular and pulp congestion with dilatation of the sinuses are nearly always noted. When exsanguination occurs, narrowing or collapse of the deeper sinuses may be present. This arises less frequently in the more fibrotic spleens. When an infection is associated, there may be greater or less infiltration of the cords with plasma cells.

The significance of the megakaryocytes and foci of extramedullary hematopoiesis requires consideration. Megakaryocytes were noted in ten cases. In five they were noted in cavernous transformation of either the portal or splenic veins; two in portal cirrhosis, and one each in hemochromatosis, phlebosclerosis of the hepatic veins and schistosomiasis. Extramedullary foci of hematopoiesis were also noted in ten cases. In five it occurred in cavernous transformation of the portal or splenic veins, in three portal cirrhoses, and one each in the cases of hemochromatosis and Bilharzia. In eight cases these two phenomena were associated, in two megakaryocytes were noted without extramedullary hematopoiesis, and in two extramedullary hematopoiesis was noted without megakaryocytes. In one case megakaryocytes were present in both liver and spleen. Apparently, these phenomena are not related to any particular cause and only occurred in the very advanced lesions of portal hypertension. This accounts for their extraordinary frequency in cavernous transformation of the portal or splenic vein, since this is the commonest cause of prolonged extrahepatic portal hypertension.

The frequent association of megakaryocytes and extramedullary hematopoiesis in the spleen permits us to regard them as simultaneous reactions. These have been frequently reported in congestive splenomegaly (57, 58, 59). Fox and Carbone (59) noted many megakaryocytes in spleens of guinea pigs ten to thirty days after ligation of the portal or splenic vessel. In other experimental reports, no mention is made of either megakaryocytes or foci of hematopoiesis. Both megakaryocytes and extramedullary erythropoiesis have been noted not only in the spleen but in other organs such as the liver, kidneys and lymph nodes in conditions in which the bone marrow function has been seriously compromised for instance in osteosclerosis (60 61) and in myelosis. These are the organs that partake in hematopoietic function during embryonic life but lose this function soon after birth, when the bone marrow assumes the entire responsibility. In many animals this function is maintained even in post embryonic life.

(62) deems these evidences of extramedullary hematopoiesis a phylogenetic reversal. This is the probable explanation for the presence of megakaryocytes and extramedullary erythrocytopoiesis in congestive splenomegaly, even though microscopic changes in the bone marrow in such individuals has not been demonstrated. The usual anemia that is associated with congestive splenomegaly may be an indication of this. The megakaryocytes in congestive splenomegaly cannot represent cells that have been swept into the circulation from the bone marrow because first, as Klemperer (4) emphasizes, they are never found in the finer arteries, second, because they are usually associated with extramedullary hematopoiesis, and third, because they are only found in prolonged obstructions with advanced lesions.

Finally, our material suggests that when cases of congestive splenomegaly are subjected to a painstaking examination, a cause for the production of the hypertension of the portal circulation is always found. The cases of "unknown" origin belong mostly to the group that have been studied after splenectomy alone. It is sometimes difficult to visualize or palpate a lesion within the portal or splenic vein at operation. As an instance, Mallory (63) reports a case of "Banti's syndrome" in which no cause was found at operation; later by microscopic examination a canalized thrombus of the splenic vein was found. This indeed occurred in one of our cases. An unknown factor is often invoked in instances of reported "recovery" after splenectomy for congestive splenomegaly. There is a grave question whether any patient ever completely "recovered" after this operation in the sense of a complete restitutio ad integrum, since a collateral circulation almost always develops in cases that come to operation, and such a collateral circulation is always potential in causing a hemorrhage into the gastrointestinal tract. Reports of cases of "recovery" even five or ten years after splenectomy are to be viewed with reservation.

SUMMARY

A morphological study of 86 spleens in various maladies associated with hypertension of the portal circulation was made. The finer morphology of these spleens was studied from the biological point of view, and the changes were traced from the earliest to the most mature phase. In this we have been aided by the simultaneous study of an accessory spleen in a few cases. The evolution is identical no matter what the cause of the portal hypertension may be. The morphological difference is merely one of degree. Other things being equal, the more distal the obstruction from the hilus of the spleen, the less intense the lesions. The duration of the portal hypertension is a much greater factor in increasing the intensity of the lesions than the height of the pressure. The morbid histology is modified by exsanguination, an associated infection or cardiac failure. Reasons have been submitted for believing that portal hypertension is the only factor that produces these lesions. The lesions in the spleen are interpreted as a venocapillary sclerosis. In the terminal stages of the morbid process, the circulation of the spleen is thus converted from an open to an almost closed one. This seriously compromises the reservoir function of the organ.

Teleologically, the venocapillary sclerosis may be viewed as a compensatory adaptation to the increased intravenous pressure. Megakaryocytes and extramedullary hematopoiesis only occur in the terminal stages and are usually simultaneous reactions. They probably represent a phylogenetic reversion. There are, no "unknown causes" for "congestive splenomegaly" when cases can be submitted to post mortem study. The term "congestive splenomegaly" is not strictly accurate, because congestion and venous hypertension are not necessarily synonymous phenomena.

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AN IMPROVED CLINICAL METHOD FOR THE ESTIMATION OF DISTURBANCES OF THE ACID-BASE BALANCE OF HUMAN BLOOD

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INTRODUCTION

The clinical conditions that are associated with disturbances of the acid-base balance of the blood are familiar to many physicians. Some of these, such as diabetic coma and nephritis, are of concern to the internist; while cases of intestinal obstruction with vomiting and of draining gastro-intestinal fistulas must be dealt with by the surgeon. Perhaps the most extreme disturbances are found in children, and the pediatrician is particularly interested in acid-base balance. But whatever the cause, and whoever bears the responsibility for management, optimal treatment depends upon two things: first an accurate diagnosis of the kind and magnitude of the disturbance, then the application of rational principles of therapy suited to the changing circumstances of the case.

The preferred method of estimating a disturbance of the acid-base balance is by the determination of the plasma CO_2 content. This is usually done on venous blood drawn and centrifuged in such a manner that uptake of oxygen by, or loss of CO_2 from, the sample is minimized.² The average normal venous plasma CO_2 content is 28.2 mM/L, with a range from 24 to 33 mM/L. If a CO_2 content of more than 33 mM/L is found, it is customarily inferred that an alkalosis is present, and if it is less than 24, a diagnosis of acidosis is made. This inference is based on the assumptions (a) that the plasma CO_2 closely parallels the bicarbonate content or "alkali reserve," (b) that other blood buffers are negligible in comparison with the bicarbonate anion and (c) that there is no respiratory abnormality. By this reasoning a low plasma bicarbonate concentration is the result of an accumulation of abnormal acids (or deficit of base) in the blood, and a high bicarbonate concentration the result of a deficit of acid (or excess of base). Hence the term "alkali reserve" applied to the plasma bicarbonate content by Van Slyke and Cullen is descriptive of its buffering ability when bases or non-volatile acids are added to or taken from the body fluids. Although Van Slyke (3) and others have been careful to point out that

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² In many hospital laboratories the original method of Van Slyke and Cullen (1) for plasma CO_2 combining power is still in use. Peters and Van Slyke state with reference to this method: "... However, it does not measure directly the concentration of either bicarbonate or total CO_2 in the circulating blood. For this reason it has been replaced by the simpler procedure of determining the CO_2 content of the true plasma..." (2). The plasma CO_2 combining power cannot be used in the nomogram presented here. For definitions of this and other factors, and of the symbols and units employed, see Table I.

these assumptions are not always true, and in some cases the plasma pH or CO_2 pressure (CO_2 tension), P_{CO_2} , must also be measured for a proper diagnosis of the disturbance, the plasma bicarbonate or CO_2 content is all too often looked upon as a necessary and sufficient index of any type of disturbance of acid-base balance. Furthermore the almost universal practice of limiting the laboratory determination to a plasma CO_2 content or CO_2 combining power has these serious drawbacks:

- (1) A mistake in diagnosis is possible.
- (2) The magnitude of the disturbance and the state of other essential factors in the acid-base balance can only be approximated.
- (3) Because the magnitude of the disturbance cannot be estimated precisely, it is difficult to evaluate the effects of different forms of treatment.

The chief purpose of this paper is to describe a nomogram for the calculation of all the factors necessary for a complete clinical evaluation of disturbances of the acid-base balance of the blood on the basis of two chemical determinations (such as plasma pH and plasma or whole blood CO_2 content) and an hematocrit determination.³ The description of the nomogram will be preceded by a brief discussion of the blood acid-base balance and followed by examples of the nomogram's potential usefulness as illustrated by data of actual cases drawn from the literature. Only the factors considered to be essential to a full clinical understanding of such cases will be considered here. For a complete discussion, both theoretical and clinical, of the acid-base balance of the blood, the reader is referred to Peters and Van Slyke (2).

FACTORS INVOLVED IN THE ACID-BASE BALANCE OF THE BLOOD

The chemical terms, symbols and units employed in this paper are given in Table I.

Ionic diagrams for human plasma, red cells and whole blood of average composition are given in figure 1. Diagrams of this type emphasize the important fact that within the physiological range of plasma pH (extreme pathological limits about 6.7 to 7.9) the variable but minute concentrations of H^+ and OH^- ions can be neglected in the balance between the sum of the cations (B^+), and the sum of the anions, the ionic concentrations being expressed in milliequivalents per liter. The anions are grouped into two main divisions. The first of these consists of the fixed acids (A^-), which are predominantly chloride ion, (Cl^-), with a much smaller fraction (X^-), which includes sulfate, lactate and other anions present only in traces in normal blood. The "fixed acids" are so called because their respective concentrations are unaffected by pH changes in the physiological range.⁴ The second main division consists of the buffer anions (HCO_3^-) and protein anions (P^-), which are altered with change in CO_2 pressure or pH. In the plasma the non- CO_2 buffer is comprised almost exclu-

³ For maximum accuracy with respect to factors of whole venous blood the oxygen saturation must also be known.

⁴ This is true of whole blood, but ignores the effect of changing pH on the Donnan (individual anion) equilibrium between plasma and red cells.

sive'y of the plasma proteins, but in the red cells organic phosphates must be reckoned in with the reduced hemoglobin and oxyhemoglobin (4). It is evident

TABLE I
Definitions of Factors and Symbols

FACTOR	SYMBOL	DEFINITION
Volumes per cent	Vols. %	Volumes of dry gas measured at 0°C. and 760 mm. pressure per 100 volumes of solution. A common unit for expressing CO ₂ or O ₂ or bicarbonate content of blood, but less desirable than the unit of mM/L.
Millimole per liter	mM/L.	1/1000 gram mole of a substance per liter of solution. CO ₂ , mM/L = (0.45)(CO ₂ , vols. %).
Millicivalent per liter	mEq/L.	1/1000 gram equivalent of an ion per liter of solution. X ⁻ , mEq/L = (X, mM/L)(valence of X ⁻).
CO ₂ tension	Pco ₂	CO ₂ pressure: pressure of dry gas, in mm. of Hg, with which the dissolved carbonic acid is in equilibrium.
Carbonic acid content	(H ₂ CO ₃)	Concentration of H ₂ CO ₃ , mM/L = f · Pco ₂ .
CO ₂ content	(CO ₂)	Concentration of total CO ₂ in solution.
CO ₂ combining power	}	CO ₂ or bicarbonate content of a solution saturated with CO ₂ at a given pressure (usually 40 mm. of Hg) or to a given pH (usually 7.40).
CO ₂ capacity		Bicarbonate concentration of plasma separated from blood and saturated at 25°C. with air containing 5.5% CO ₂ . Definition and method of Van Slyke and Cullen (1).
Plasma CO ₂ combining power		
Bicarbonate	(HCO ₃ ⁻)	Bicarbonate concentration, mEq/L. In this paper carbamino CO ₂ is included in bicarbonate. (HCO ₃ ⁻) = (CO ₂) - f · Pco ₂ .
Base	B ⁺	Any cation in biological fluids. (This biochemical usage is counter to the modern physicochemical definition of base.)
Total base	(B ⁺)	Sum, in mEq/L, of concentrations of individual cations in blood.
Buffer base	(BB ⁺)	Base equivalent to the sum of buffer anion concentrations (including HCO ₃ ⁻) in mEq/L.
Fixed acid	(A ⁻)	Sum, in mEq/L, of concentrations of individual non-buffer anions.
Non-CO ₂ buffer	(P ⁻)	Sum of buffer anion concentrations exclusive of HCO ₃ ⁻ , in mEq/L, in blood, plasma or red cells.
Oxygen saturation	S	Per cent of total hemoglobin in the form of oxyhemoglobin.
Oxygen unsaturation	U	U = 1 - S/100
Hematocrit	Vc	Volume fraction of packed red cells in whole blood.

Subscript s denotes plasma or serum, subscript e denotes red cell, subscript b denotes whole blood.

from the diagrams that part of the total base (B⁺) is equivalent to the fixed acids (A⁻), while the remainder is equivalent to the sum of (HCO₃⁻) and (P⁻).

This second fraction is defined as the "buffer base" (BB^+). This may be expressed in two ways:

$$(\text{BB}^+) = (\text{HCO}_3^-) + (\text{P}^-) \quad \text{eq. (1)}$$

$$(\text{BB}^+) = (\text{B}^+) - (\text{A}^-) \quad \text{eq. (2)}$$

The buffer base is most important to this consideration of disturbances of acid-base balance because it embraces in a single factor the net effect of several

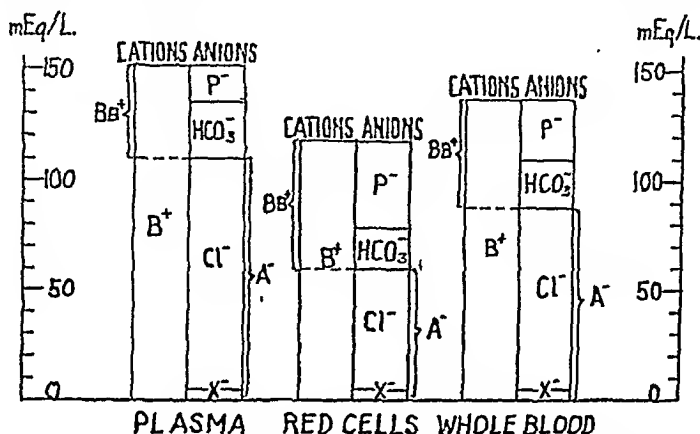


FIG. 1. ACID-BASE (IONIC) DIAGRAMS FOR NORMAL ARTERIAL OR FULLY OXYGENATED HUMAN BLOOD

CO_2 pressure, $\text{Pco}_2 = 41$ mm. $\text{pH}_s = 7.41$. Hematocrit, $\text{Vc} = 0.45$.

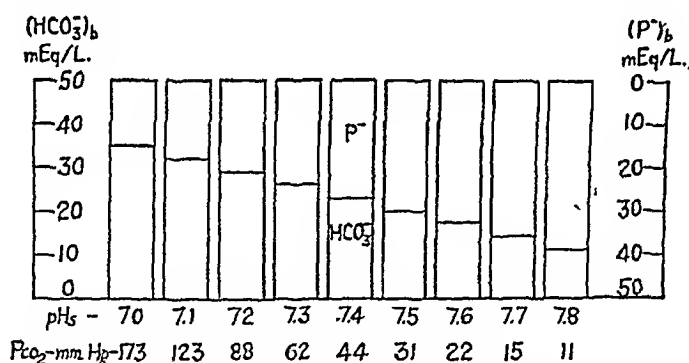


FIG. 2. EFFECT ON BUFFER ANIONS OF VARYING CO_2 PRESSURE, Pco_2 AND pH .

Oxygenated whole blood. Hematocrit, $\text{Vc} = 0.45$. Whole blood buffer base $(\text{BB}^+)_b = 50$ mEq/L.

variables: the concentrations of all the individual ions that are included in (B^+) and (A^-) . These ions are the ones altered only by non-respiratory or "metabolic" changes in the body—changes in intestinal absorption, in secretion and in renal excretion. A decrease in buffer base (BB^+) is produced by a decrease in (B^+) , or an increase in one or more of the fixed acids (A^-) . This necessarily results in smaller values for (HCO_3^-) and (P^-) . An increase in (BB^+) is correspondingly brought about by an increase in (B^+) or a decrease in (Cl^-) , and implies higher values of (HCO_3^-) and (P^-) .

Figure 2 shows (P^-) and (HCO_3^-) plotted in the manner of the ionic diagram at intervals of 0.1 plasma pH , for oxygenated blood of a hematocrit of 0.45 and

a whole blood buffer base of 50 milliequivalents per liter. The reciprocal variation of (HCO_3^-) and (P^-) and the constancy of (Bn^+) as the CO_2 pressure is altered are clearly shown. Respiratory changes, by affecting the CO_2 pressure, may produce a very abnormal bicarbonate content or CO_2 content, of either whole blood or plasma, in the presence of a normal buffer base.

It is obvious from figure 1 that, expressed on a volume basis, the individual and total ionic concentrations in the red cell are quite different from those in the plasma. Hemoglobin is a more effective buffer than the plasma proteins, and (P^-) is considerably higher in the red cell than it is in plasma. In whole blood the ionic pattern will depend on the respective ionic patterns and amounts of the two phases, plasma and red cells, present. Although pH and usually CO_2 content are determined experimentally on plasma, it is better for present purposes to use whole blood rather than plasma buffer base because the latter is affected by the "chloride shift" between plasma and red cells as the pH is altered, and because all the blood buffers must be taken into account. From the foregoing it is apparent that packed red cell volume or hematocrit, V_c , is an essential factor in the use of the nomogram.

Plasma or whole blood bicarbonate is not measured directly but must be calculated from total CO_2 concentration and carbonic acid concentration as shown in Table I. P_{CO_2} is seldom measured directly on blood; it can be calculated from the pH, and $(\text{CO}_2)_s$ of plasma by means of the familiar Henderson-Hasselbalch equation:

$$\text{pH}_s = \text{pK}_s + \log \frac{(\text{HCO}_3^-)_s}{(\text{H}_2\text{CO}_3)_s} \quad \text{eq. (3)}$$

$$\text{pH}_s = \text{pK}_s + \log \frac{(\text{CO}_2)_s - f_s \text{P}_{\text{CO}_2}}{f_s \text{P}_{\text{CO}_2}} \quad \text{eq. (4)}$$

P_{CO_2} (CO_2 pressure) is very rapidly altered by changes in lung ventilation, and it can be looked upon as the primary factor in respiratory disturbances of acid-base balance, just as $(\text{Bn}^+)_s$ (buffer base) is the primary factor in metabolic disturbances. The P_{CO_2} of arterial blood is practically identical with and is controlled by the alveolar P_{CO_2} .

Blood oxygen saturation is the last factor that must be included in this abbreviated description of the acid-base balance. Oxyhemoglobin has stronger acidic properties than does reduced hemoglobin; in figure 2 the value of (P^-) at each pH would be about 4 milliequivalents lower if the blood were completely reduced instead of oxygenated. Blood with an oxygen saturation of 90% or over can be considered as fully oxygenated blood for purposes of obtaining the state of the acid-base balance by means of the nomogram. The advantage of using arterial or capillary (finger) blood is that the oxygen saturation can be assumed to be 97 per cent except in rare cases of arterial anoxemia. With venous blood, however, the oxygen saturation may vary from 35 per cent to 75 per cent, at least; whole blood CO_2 and buffer base figures must be corrected for the degree of oxygen unsaturation for the most precise results.⁵

⁵ Venous blood of arterial properties can be obtained by heating the arm in water at 45°C. for several minutes prior to vein puncture (5).

In summary, then, five factors are necessary for an adequate description of the acid-base balance of arterial blood: (1) hematocrit; (2) blood or alveolar P_{CO_2} ; (3) plasma pH; (4) whole blood buffer base concentration; (5) plasma or whole blood CO_2 content. For partly reduced blood a sixth factor, the oxygen

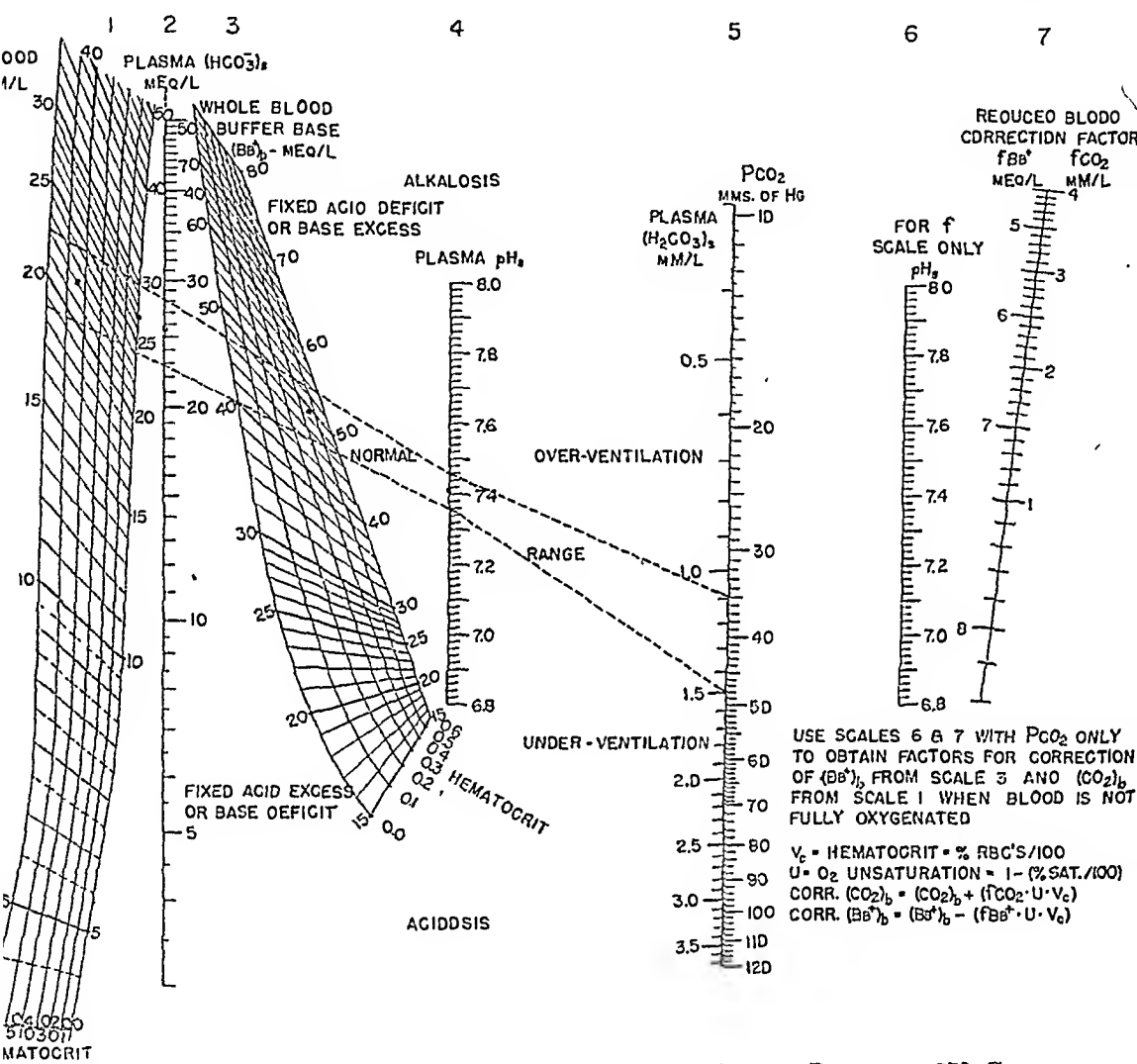


FIG. 3. NOMOGRAM FOR THE ACID-BASE BALANCE OF HUMAN BLOOD AT 37° C.

For oxygenated blood (scales 1 to 5) a straight line through points given on two of the scales intersects the remaining scales at simultaneously occurring values of the other variables. The position of the line indicates the kind and magnitude of any disturbance of the acid-base balance. The normal range is for arterial, not venous blood. For details of use, see text.

saturation, is required for precise results. Other factors, such as plasma bicarbonate and carbonic acid concentrations, may be directly derived from the foregoing but are not necessary for clinical purposes.

DESCRIPTION OF THE NOMOGRAM AND ITS USE

The nomogram is shown in figure 3. It consists of seven scales, which are, reading from left to right (1) whole blood CO_2 content and plasma CO_2 content,

(2) plasma bicarbonate, (3) whole blood buffer base (Bb^+)_b, (4) plasma pH, (5) CO_2 pressure and plasma carbonic acid concentration, and (6) an auxiliary plasma pH scale for use with (7) a scale of correction factors for (CO_2)_b and (Bb^+)_b in reduced blood. The first five scales constitute the principal part of the nomogram. Scales 2, 4 and 5 are for plasma constituents, are unilinear and are a theoretically exact portrayal of the Henderson-Hasselbalch equation (equation 3). Scales 1 and 3 are multilinear, or in the form of grids, with a separate scale for hematocrit values at intervals of 0.1 from $V_c = 0$ (plasma) to a maximum of $V_c = 0.6$ in whole blood; they were constructed for oxygenated blood. In using these scales for whole blood one must interpolate between the hematocrit as well as the (CO_2)_b or (Bb^+)_b lines in locating a given point. Given any two of the experimentally obtainable variables, CO_2 content (whole blood or plasma), plasma pH, or alveolar (arterial) CO_2 pressure, P_{CO_2} , together with the hematocrit of the blood one can locate two points on the appropriate scales and draw a straight line through them. This line intersects the remaining scales at simultaneously occurring values of the other variables, and gives an abbreviated but adequate characterization of the state of acid-base balance of the blood at the time it was drawn. Four examples will be presented to illustrate the use of the nomogram in four possible situations under which the data may be given. The correction of whole blood CO_2 and (Bb^+)_b as shown in the last two examples, where venous or incompletely oxygenated blood is analyzed, may be omitted in clinical practice if one is willing to accept an error in the buffer base of about 1 mEq/L and small errors in the other factors.

(a) Plasma data alone. Given: (CO_2)_s = 11.1 mM/L and $\text{pH}_s = 7.59$. Procedure: Locate the point 11.1 on the right-hand edge of the scale 1 grid. (Scale 1 is labeled "whole blood CO_2 ", but the right-hand scale, where the hematocrit is zero, represents plasma.) Locate the point 7.59 on Scale 4. Draw a line^a through these two points and read off the CO_2 pressure on Scale 5, the plasma buffer base on the Scale 3 grid (left-hand edge where $V_c = 0$) and plasma bicarbonate on Scale 2. Since only plasma data are under consideration the results are correct regardless of the hematocrit or oxygen saturation of the blood.

Results: (HCO_3^-)_b = 10.9 mEq/L. (Bb^+)_s = 29.5 mEq/L. P_{CO_2} = 11.8 mm of Hg.

(b) Oxygenated whole blood. Given: Finger blood, (CO_2)_b = 28.8 mM/L, $\text{pH}_s = 7.48$, $V_c = 0.48$. Procedure: Locate the point 28.8 on a line 0.8 of the distance from $V_c = 0.4$ to $V_c = 0.5$ in the whole blood CO_2 grid (Scale 1) and the point 7.48 on Scale 4. Draw a line connecting the points and read off P_{CO_2} on Scale 5 and (Bb^+)_b from the Scale 3 grid, again interpolating between $V_c = 0.4$ and $V_c = 0.5$. (CO_2)_s, (HCO_3^-)_s and (Bb^+)_s may also be read off if desired.

Results: (Bb^+)_b = 58.5 mEq/L. P_{CO_2} = 48 mm of Hg.

(c) Partially oxygenated blood, plasma data. Given: (CO_2)_s = 11.1 mM/L,

^a A plain transparent rule with a straight line etched on the under surface is of great assistance in locating points accurately on the nomogram scales.

$pH_s = 7.59$, $V_c = 0.59$, Oxygen saturation = 80 per cent. To be calculated: P_{CO_2} , F_{CO_2} , F_{BB^+} , corrected $(CO_2)_b$ and corrected $(BB^+)_b$. Procedure: Locate the given points as in example 1 and read off $P_{CO_2} = 11.8$ mm, and provisional values of $(CO_2)_b$ and $(BB^+)_b$, interpolating between $V_c = 0.5$ and $V_c = 0.6$. Then with 11.8 on Scale 5, and 7.59 on Scale 6 read off from Scale 7 $F_{CO_2} = 0.9$ and $F_{BB^+} = 7.6$. The oxygen unsaturation $U = 1 - \frac{80}{100} = 0.2$. Calculate the corrections for $(CO_2)_b$ and $(BB^+)_b$ by multiplying each factor by $U \times V_c$. CO_2 correction = $(0.9)(0.2)(0.59) = 0.1$ mM. BB^+ correction = $(7.6)(0.2)(0.59) = 0.9$. Add the CO_2 correction to the value read from Scale 1 and subtract the BB^+ correction from the value read from Scale 3.

Results: Corrected $(BB^+)_b = 45 - 0.9 = 44.1$. Corrected $(CO_2)_b = 8.2 + 0.1 = 8.3$ mM/L. Note that the deviation of $(BB^+)_b$ from the lower limit of normal is not so great as the deviation of $(BB^+)_s$. For most clinical purposes it may be sufficient to take $(BB^+)_b$ directly from the nomogram without applying the correction for oxygen saturation, which is seldom greater than 2 mEq/L.

(d) Partially oxygenated blood, $(CO_2)_b$ one of data. Given: $(CO_2)_s = 31.2$ mM/L, Alveolar $P_{CO_2} = 62.3$ mm, $V_c = 0.59$, O_2 saturation = 89 per cent. To be calculated: pH_s , F_{CO_2} , F_{BB^+} , oxygenated $(CO_2)_b$, corrected $(BB^+)_b$. Procedure: Locate $(CO_2)_b$ on Scale 1 grid and P_{CO_2} on Scale 5 and find a preliminary pH_s of 7.41 on Scale 4. With $P_{CO_2} = 62.3$ and $pH_s = 7.41$ on Scale 6, read off $F_{CO_2} = 2.8$ from Scale 7. Oxygen unsaturation $U = 1 - \frac{89}{100} = 0.11$. CO_2 correction = $(2.8)(0.11)(0.59) = 0.2$ mM. This correction is subtracted from the given $(CO_2)_b$, since, at this necessary intermediate step, we must convert from reduced to oxygenated blood, instead of from oxygenated to reduced blood, as in the previous example. Oxygenated $(CO_2)_b = 31.2 - 0.2 = 31.0$ mM/L. This is the $(CO_2)_b$ this blood would have had if fully saturated with respect to oxygen. Now return to the main part of the nomogram and with $P_{CO_2} = 62.3$ and $(CO_2)_b = 31.0$ read off $(BB^+)_b = 60.5$ mEq/L and $pH_s = 7.41$. The CO_2 correction was small, so the pH_s is the same as on the initial trial, and the same calculation of CO_2 correction applied with the usual + sign gives the original $(CO_2)_b$ of 31.2 mM/L. The factor for (BB^+) is 5.7 mM/L and the correction = $(5.7)(0.11)(0.59) = 0.5$. Corrected $(BB^+)_b = 60.5 - 0.5 = 60.0$ mEq/L.

Results: $(BB^+)_b = 60.0$ mEq/L. $pH_s = 7.41$. Here, as in example (c), the corrections for oxygen unsaturation can be ignored when maximum precision is not necessary.

CONSTRUCTION OF THE NOMOGRAM

The nomogram has been constructed from data in the literature on the buffer curves of human oxygenated and reduced whole blood and plasma (6), and the relation of plasma to whole blood CO_2 content (7) for blood of varying hematocrit or total hemoglobin content. Average values have been assumed for protein concentration in the plasma (72 grams per liter of plasma) and for hemoglobin

concentration in the red cell (20 mM of O_2 capacity per liter of cells). Deviation from these figures will affect the buffer curves of the blood, and hence the accuracy of the nomogram. However, the wide variation to which plasma protein concentration is subject introduces a small error because of the relatively small buffer value of the plasma proteins. The hemoglobin concentration in the red cell has a rather narrow normal range of 18.7 to 21.2 mM/L; the upper limit is seldom exceeded, and lower values occur principally in iron-deficiency type anemias. The red cell organic phosphates, which have been shown to change markedly in certain disturbances, are a factor in the buffer curves of normal blood as used in the construction of the nomogram. All these assumptions introduce minor errors in the values obtained by use of the nomogram, but do not affect significantly the clinical interpretation of the data.

The errors inherent in the construction of the nomogram and in the reading of the scales can be stated more exactly. If care is used in locating points and the line of acid-base balance, the reading error does not exceed ± 2 per cent and is generally less than ± 1 per cent. The P_{CO_2} , plasma pH and bicarbonate scales are theoretically exact, while the whole blood CO_2 , plasma CO_2 , buffer base and correction scales are approximations of the data on which they are based. Values read from the latter scales agree with corresponding ones calculated from the basic formulas within ± 3 per cent, except at the extreme ends of the scales, i.e., outside a pH range of 7.0 to 7.8, where somewhat greater deviations may be encountered. Errors of this magnitude are negligible in clinical work.

DISTURBANCES OF THE ACID-BASE BALANCE

The normal average values of the different factors have been indicated by small solid squares on the scales of the nomogram, and the normal range by broken lines intersecting the scales. These values have been selected after a careful review of data on arterial and finger blood and alveolar air composition. The range selected includes about 95 per cent of $(CO_2)_b$, $(CO_2)_a$ and pH_a values in arterial blood of normal individuals at rest, and about 90 per cent of arterial or alveolar P_{CO_2} values. A greater variability is reported in the extensive series of finger blood determinations by Shock and Hastings (8), but the activity of these subjects was not controlled.

The nomogram is a representation of the mathematical fact that for blood of any given hematocrit and oxygen saturation all the factors in the acid-base balance are determined when any two of them are known. The state of acid-base balance is shown by a straight line, which is fixed by *any two* of its intersection points on Scales 1 to 5. From a mathematical standpoint any two of the variables may be used as independent ones to fix the line, and the values of the other variables are necessarily dependent on them. From the experimental standpoint it is practicable to determine some but not all of the possible combinations of the different factors represented. But from the physiological standpoint the two factors that are primarily and directly subject to the action of the regulatory and metabolic activities of the body are the *whole blood buffer base*, $(Bb^+)_b$, and

the arterial or alveolar CO_2 pressure, P_{CO_2} . In this sense the whole blood and plasma CO_2 contents, the plasma bicarbonate content and pH are dependent variables. To classify disturbances of acid-base balance it appears advantageous to use the two "physiologically independent" variables and they are so employed here. The classification given in Peters and Van Slyke (2) is similar in outline, but differs somewhat in details of nomenclature and does not provide numerical values for the buffer base. *Any classification in reality merely supplements the use of the nomogram, which contains all the information required to identify a given disturbance, if the fundamental physiological processes involved are understood.*

For purposes of illustration Table II has been compiled from cases of acid-base disturbance reported in the literature. The diagnosis and reference are given in column 2, and the analytical data in columns 4 to 8. The values of P_{CO_2} and $(BB^+)_b$, as obtained from the nomogram, are in columns 9 and 10, and the deviations of these factors from their respective normal means, in columns 11 and 12. The cases are arranged in sequence according to the direction of the disturbance in $(BB^+)_b$ and P_{CO_2} (as evidenced by the + and - signs in columns 11 and 12), and their classification is listed in column 13. If the line of acid-base balance for each case in Table II is plotted from the analytical data, the use of the nomogram in diagnosis of acid-base disturbances will be apparent.

METABOLIC ALKALOSIS AND ACIDOSIS

Since disturbances of the buffer base are the most common clinically, this factor will be considered first. An *increase* in the whole blood buffer base is produced by a *fixed acid deficit* or *base excess*. This may be termed a *metabolic alkalosis*. The upper part of Scale 3 grid, above the upper, broken, normal line on the nomogram is labeled "fixed acid deficit or base excess." A line of acid-base balance that crosses this part of the buffer base grid represents a disturbance involving a deficit of fixed acid or excess of base. A deficit of fixed acid occurs with loss of chloride from plasma and extracellular fluid in persistent vomiting, particularly as a result of high intestinal obstruction (case 2, Table II) or in continuous loss of stomach contents by gastric fistula or suction drainage. An excess of base, on the other hand, is produced by ingestion of alkalizing salts, such as the bicarbonate, lactate or citrate of sodium or potassium (cases 1 and 3). The anions of these salts are excreted or metabolized rapidly, leaving the equivalent amount of cation to augment the concentration of whole blood buffer base.

A *decrease* in buffer base is conversely the result of a *fixed acid excess* or *base deficit*, as indicated on the nomogram next to the lower part of Scale 3 grid. This is often called a *metabolic acidosis*. The anion involved in the increase in fixed acid may be Cl^- , SO_4^{--} , HPO_4^{--} , an organic radicle such as lactate or acetoacetate, or an abnormal radicle such as NO_3^- . Certain salts of ammonia and the alkaline earths are acidifying (Case 11), in the case of the former because the ammonia is rapidly converted to urea in the liver, and in the latter because the cation appears to be poorly absorbed from the gastro-intestinal tract. Apart from these exogenous acids lactic acid may be produced in the body in large quantities during severe exercise, anoxia, hemorrhage or ether anesthesia, and

TABLE II

Examples of Acid Base Disturbances

Analytical data are taken from the references cited in column 2. Where analytical data are lacking for the factors in columns 4 and 5, assumed values are indicated by parentheses. In the calculation of ΔP_{CO_2} and $\Delta(Bu^+)_{\text{b}}$ in columns 11 and 12, normal values of arterial P_{CO_2} and $(Bu^+)_{\text{b}}$ are taken from the nomogram at the analytical V_c given in column 5, for venous blood a normal mean P_{CO_2} of 46.5 mm is assumed.

CASE	ETIOLOGY AND REFERENCE	BLOOD SAMPLE*	ANALYTICAL DATA					NOMOGRAM FACTORS				CLASSIFICATION OF DISTURBANCE
								Values		Deviations from normal mean		
			O ₂ sat	V _c	(CO ₂) _b	(CO ₂) _a	pH _a	P _{CO₂}	(B _u ⁺) _b	ΔP _{CO₂}	Δ(B _u ⁺) _b	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
			%		mM/L	mM/L		mm	mEq/L	mm	mEq/L	
1	Experimental NaHCO ₃ ingestion and over-ventilation (9)	HA	(95)	0.51		30.0	7.64	27.7	58	-13.8	+8	Mixed alkalosis (primary metabolic and primary respiratory)
2	Intestinal obstruction (10)	V	(60)	(0.40)		39.5	7.54	45.5	60.5	N.R.†	+12.5	Metabolic alkalosis
3	Experimental NaHCO ₃ ingestion (11)	F	(97)	0.48	23.8		7.48	48.0	58.5	+7.5	+8.5	Metabolic alkalosis with slight secondary respiratory acidosis
4	Hypertensive cardiovascular renal disease (12)	V	(60)	(0.40)		38.4	7.41	55.8	54.5	+10	+6.5	Mixed disturbance metabolic alkalosis and respiratory acidosis
5	Emphysema 'fixed chest' (13)	A	89	0.59	31.2		7.41†	62.3§	60	+20.8	+9	Mixed disturbance primary respiratory acidosis with secondary metabolic alkalosis
6	Fever (T 104°) due to grippe (14)	V	(60)	(0.40)		24.2	7.53	28.5	48	-18	N.R.†	Respiratory alkalosis
7	Experimental inhalation of CO ₂ (11)	F	(97)	0.48	25.3		7.25	65.0	48	+14.5	N.R.†	Respiratory acidosis
8	Normal man acclimatized to altitude of 19 000 feet (15)	A	85	0.55	14.0		7.43†	26.0§	42.5	-15.5	-8	Mixed disturbance primary respiratory alkalosis with secondary metabolic acidosis
9	Acute encephalitis and ketosis (16)	A	80	0.59		8.3	7.59	11.8	44	-23.7	-7	Mixed disturbance primary respiratory alkalosis and primary metabolic acidosis
10	Renal failure carcinoma of kidney (10)	V	(60)	(0.40)		4.9	7.01	14.5	18.5	-32	-29.5	Metabolic acidosis with secondary respiratory alkalosis
11	Experimental NH ₄ Cl ingestion (11)	F	(97)	0.42	17.2		7.25	44.2	40	N.R.†	-8	Metabolic acidosis
12	Therapeutic CO ₂ administration comatose mental patient (17)	A	99	0.50	24.6		6.89	132	32	+91	-18	Mixed acidosis (primary respiratory and primary metabolic)

* A, arterial blood; F, finger blood; HA, venous blood from heated arm; V, venous blood

† N.R. factor within normal range

‡ pH_a calculated from nomogram, P_{CO_2} being one of analytical data.

§ P_{CO_2} determined on end-expiratory alveolar air sample

the keto acids, β -hydroxybutyric and aceto-acetic, in uncontrolled diabetes and in starvation. In terminal nephritis or any condition associated with failure of kidney function, retention of phosphates and sulfates results in a fixed acid excess (case 10). A deficit of total base relative to fixed acid is seen in the severe diarrheas of infancy, in cholera and dysentery and in fistulas of the intestinal or biliary tract. The intestinal and biliary secretions, in contrast to the gastric juice, contain relatively more base than fixed acid.

RESPIRATORY ALKALOSIS AND ACIDOSIS

The clinical conditions that have been mentioned above all involve a primary change in the whole blood buffer base. By primary we mean that the change is induced by physiological processes such as absorption, excretion or metabolism, processes directly influencing the concentration of whole blood buffer base. In a similar manner we may speak of a primary change, by altered respiratory activity, in the arterial CO_2 pressure, P_{CO_2} , the second of the two "independent" variables of the acid-base balance. Most disturbances of the acid-base balance produced by a primary change in one of these two factors will manifest, if they persist long enough, a secondary change in the other factor. Such a change is always in the direction of effecting a more proportionate distribution of the disturbance among the different variables, and is the result of physiological regulatory mechanisms set in to operation by the original disturbance. For example, a primary fixed acid excess, unless of very mild degree, is almost always accompanied by a secondary decrease in P_{CO_2} . Let us suppose the arterial whole blood buffer base to be lowered to 30 mEq/L, (Scale 3), with $V_c = 0.40$ and with a normal P_{CO_2} of 40 mm (Scale 5). The plasma pH_s would then be 7.10 (Scale 4) and $(\text{CO}_2)_s$, 13.1 mM/L (Scale 1). With a secondary decrease in P_{CO_2} to 27 mm the pH_s becomes 7.18 and $(\text{CO}_2)_s$ 10.6 mM/L. It will be observed that this secondary change in P_{CO_2} is associated with an increased deviation of $(\text{CO}_2)_s$ from normal, but with a smaller deviation of pH_s . A reduction of the pH_s deviation always accompanies a secondary change in (BB^+) or P_{CO_2} .

In the case of secondary change in $(\text{BB}^+)_b$ the regulatory mechanism is selective excretion and production of acid or alkaline urine by the kidneys (cases 5 and 8); in the case of arterial P_{CO_2} it is control of ventilation rate by reflex and direct action on the respiratory center (cases 3 and 10). Such mechanisms are homeostatic with respect to the acid-base balance of the blood, but we have avoided the use of the term "compensatory" change because the latter is customarily taken to signify a deliberate attempt on the part of the organism to return the plasma pH to normal. In a case of fixed acid excess with secondary decrease in P_{CO_2} there is usually some degree of acidosis still present; there is no evidence to suggest that a state of normal pH_s and low P_{CO_2} is better tolerated by the body than one of low pH_s and normal P_{CO_2} , and hence that the one state should be called compensated and the other uncompensated. We consider it better to speak of the change in P_{CO_2} or $(\text{BB}^+)_b$ as primary or secondary. There may be a primary change in both factors simultaneously and the clinical circumstances of the case must be relied upon to tell whether the deviations determined on the nomogram are primary or secondary.

While primary clinical disturbances of P_{CO_2} are less common than disturbances of $(BB^+)_b$, primary physiological changes are frequent. Arterial P_{CO_2} is decreased in exercise, in relative anoxia or in any state with a considerable increase of metabolic rate; it is increased during sleep. On the nomogram the upper part of the P_{CO_2} scale, with pressures less than 35 mm, has been designated "over-ventilation," and the lower part of the scale, with P_{CO_2} greater than 48 mm, "under-ventilation." The alveolar or arterial P_{CO_2} can also be raised by adding CO_2 to the inspired air, in which case over-ventilation may be seen associated with a high P_{CO_2} .

A *respiratory alkalosis* may be defined as a primary decrease in P_{CO_2} . Such a decrease is found in most cases of fever or hyperpyrexia due to any cause (case 6), in cases of encephalitis (case 9), brain tumor or other neurological conditions producing an irritation of the respiratory center, in hysterical over-ventilation, and in some cases of traumatic shock and cardiac decompensation.

A primary increase in P_{CO_2} , or *respiratory acidosis* is encountered in some cases of pulmonary disease (case 5), in cases of narcotic or anesthetic depression of the respiratory center, in respiratory paralysis due to convulsions, electric or metrazol shock, or to curarization (case 12), and in a few cases of cardiac decompensation. It can also be produced by inhalatory administration of gas mixtures containing CO_2 (case 12). Cases of pneumonia and other lung conditions severe enough to produce cyanosis and anoxemia seldom interfere with CO_2 exchange because CO_2 is so much more diffusible than oxygen. It is only in cases of emphysema with rigid chest (case 5), where total air exchange is limited to a minimum, or cases of the most extensive destruction of alveolar tissue, that a build-up of arterial P_{CO_2} occurs.

MIXED ³DISTURBANCES

As stated previously, a primary change in one of the independent factors, whole blood buffer base or CO_2 pressure, is often accompanied by a secondary change in the other factor. A primary metabolic acidosis, with a secondary respiratory alkalosis, such as that described in the preceding section, would be an example. Such combinations are all *mixed disturbances* of the acid-base balance in which the deviations of $(BB^+)_b$ and P_{CO_2} tend to produce opposing changes in pH. Similar combinations are also encountered in which both independent factors have undergone a primary change. In all of these cases (cases 3 to 5 and 7 to 9) the pH disturbance, if any, is given unambiguously by use of the nomogram, but often cannot be predicted from the name of the mixed disturbance without the nomogram. A final group of mixed disturbances is that in which both factors tend to produce the same deviation in pH—the group comprising *mixed acidosis* (case 1), and *mixed alkalosis* (case 12), (each a primary metabolic and a primary respiratory disturbance); there is no uncertainty with regard to the pH shift here.

On the nomogram "acidosis" and "alkalosis" have been placed at the appropriate ends of pH_s scale 4. Unqualified, these terms, "acidosis" and "alkalosis," are meant to indicate an abnormal pH_s value, and this alone. When qualified by "metabolic," however, they refer to changes in $(BB^+)_b$, and when qualified by

"respiratory," to changes in P_{CO_2} . The pH deviation is then indicated by the acidosis or alkalosis of the primary factor, with the exception of certain mixed disturbances, where, as indicated above, the nomogram may be required to fix the pH.

An examination of the CO_2 values in Table II will disclose that a normal CO_2 content may exist in the presence of a severe acidosis or alkalosis of the mixed type, and that both an increase and decrease in $(BB^+)_b$ may be associated with either a high or a low CO_2 content. The disadvantages of using CO_2 content alone as an index of metabolic acidosis or alkalosis are apparent.

OTHER APPLICATIONS OF THE NOMOGRAM

The nomogram can be used in many ways to illustrate aspects of the acid-base balance of the blood: the CO_2 dissociation curves of whole blood, true plasma or separated plasma; the relationship of CO_2 combining power and CO_2 content; the effects of anemia on the buffer properties of blood; the chloride shift between plasma and red cells as a result of pH changes. But to the clinician the most important use, next to that of making the initial diagnosis, is in following changes in acid-base balance in response to treatment. One clinical example has been selected for this purpose: a case of diabetic coma reported by Bock, Field and Adair (18).

This is the case of a 14-year old boy with uncontrolled diabetes of one year's duration, admitted with the typical signs of diabetic coma. Arterial blood was used for the determination of whole blood CO_2 content, P_{CO_2} , by interpolating on measured CO_2 dissociation curves (in some specimens alveolar P_{CO_2} was also determined). No hematocrits are reported, and only two oxygen capacity values are given, so we have assumed a value of 0.40 for V_c after the dehydration was corrected. The data are summarized in figure 4 together with the calculated pH_s and $(BB^+)_b$, all values being plotted according to the date and time the blood sample was taken. The values of pH_s from the nomogram are higher than those given by Bock, Field and Adair because the latter values have been calculated from whole blood CO_2 and P_{CO_2} data without the proper corrections having been applied.

Treatment consisted of orange juice by mouth, intramuscular saline solution, insulin and alkali in the form of sodium bicarbonate. No alkali was given until six hours after admission, when sodium bicarbonate was started by rectal infusions because of the small change in $(CO_2)_b$ and P_{CO_2} in response to other treatment. Afterward the sodium bicarbonate was given by mouth. It was stopped and resumed on two occasions because the $(CO_2)_b$ fell each time it was discontinued. The total amount of alkali and duration of its administration for each of these periods are given in figure 4. The changes in buffer base and in the other factors in response to the sodium bicarbonate administered are shown very clearly.

This case demonstrates a feature of diabetic acidosis that has not received sufficient attention, despite its being noted as long ago as 1917 by Stillman, Van Slyke, Cullen and Fitz (19): the return of buffer base toward normal is relatively

more complete and prompt than the return of P_{CO_2} . The authors just cited report a relatively low alveolar P_{CO_2} in five of ten cases of diabetic acidosis, when the CO_2 combining power is only moderately reduced. In the case given here the persistence of the low P_{CO_2} has resulted in an alkalosis after the third day as the pH_a varies between 7.44 and 7.58 after this date. This is contrary to what one would expect if the change in P_{CO_2} were purely secondary to the acid excess; respiratory responses to pH change are very rapid. It seems clear from these

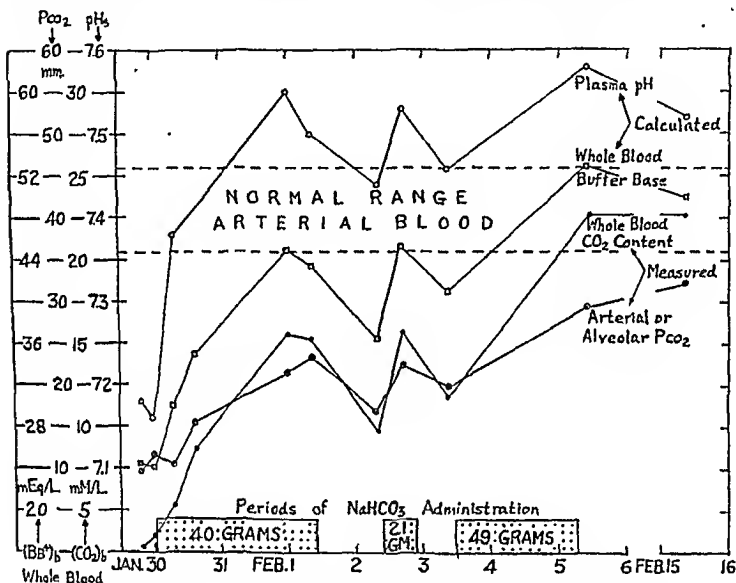


FIG. 4. COMPLEX ACID-BASE PATHWAY IN RECOVERY FROM DIABETIC COMA, ILLUSTRATING MIXED NATURE OF THE ACID-BASE DISTURBANCE: PRIMARY METABOLIC ACIDOSIS AND PRIMARY RESPIRATORY ALKALOSIS

Persistence of over-ventilation and low CO_2 pressure result in marked alkaline shift in pH_a following $NaHCO_3$ therapy. Case IV reported by Bock, Field and Adair (18).

and other cases in the literature (20, 21) that the disturbance of acid-base balance in uncontrolled diabetes is the product not only of an acid excess but also of a primary overventilation. The latter is possibly due to the accumulation of acid metabolites in the respiratory center, which would be quite in keeping with the much slower return of the respiratory factor to normal in response to treatment. In this case the administration of alkali in the later stages of treatment has indeed raised the buffer base and CO_2 content toward normal, but it has exaggerated the rise of pH_a above normal. The drop in buffer base and $(CO_2)_b$ on February 2 (figure 4) may have been secondary to the persistent hyper-

ventilation rather than a recurrence of the primary accumulation of keto-acids in the blood. Continued administration of alkali had the effect of raising the pH_s to an abnormal range again. These data illustrate clearly the errors of interpretation that may result from using the CO_2 content alone as a guide to alkali therapy in diabetic acidosis.

The fact that two chemical analyses are required to obtain the state of acid-base balance does not imply the necessity of larger blood samples from the patient. The determination of the pH_s , whole blood CO_2 content and hematocrit may be carried out on as little as 0.1 ml of blood (22). This provides the data, necessary and sufficient, to permit estimation of the CO_2 pressure and whole blood buffer base from the nomogram. Since the method is a micro one, finger blood can be used and serial determinations made on the equivalent of arterial blood without the disadvantage involved in multiple arterial punctures.

SUMMARY

1. The plasma CO_2 content or CO_2 combining power, by itself, is always incomplete and occasionally deceiving as an index of the state of acid-base balance of the blood.

2. The principal factors involved in an adequate clinical description of the acid-base balance are the CO_2 content of whole blood or plasma, the hematocrit, the plasma pH , the whole blood buffer base, the CO_2 pressure and the oxygen saturation. For blood of a given hematocrit and oxygen saturation two of these factors are necessary to determine the others.

3. A nomogram is described that provides the values of the remaining factors in arterial blood when two of the factors and the hematocrit are known; corrections are also furnished for venous or incompletely oxygenated blood.

4. The nomogram is used to classify disturbances of acid-base balance on the basis of the levels of whole blood buffer base and arterial CO_2 pressure. The importance of a complete diagnosis of such disturbances is discussed and illustrated by the analysis of reported cases, including the changes that occur during recovery from diabetic acidosis.

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APPENDIX

CONSTRUCTION OF THE NOMOGRAM

The nomogram was first applied to the problem of the physico-chemical equilibrium of the blood by L. J. Henderson, and a discussion of some of the relevant mathematical features of the nomogram will be found in his book entitled "Blood, A Study in General Physiology." The necessary condition for the construction of a nomogram relating a number of variables u , v , w , which are functions of two independent variables x and y is that the following equation must be satisfied:

$$y = m_x x + b_x$$

where m_x and b_x are single-valued at any value, n , of z , which represents u , v , or w . In the case of the blood the selection of the independent variables and their

form of inter-relation are matters of convenience. Here it has been found most convenient to let y equal whole blood CO_2 content, or whole blood buffer base concentration, x , the CO_2 pressure, and z , the plasma pH. Graphs were constructed for these relations in oxygenated and in reduced blood, with several different values assumed for the hematocrit, V_c . Scales 2 and 5 (logarithmic representations of plasma bicarbonate concentration and P_{CO_2}) were drawn on the nomogram first, and the linear plasma pH scale, 4, was then fixed by the Henderson-Hasselbalch relation. From the graphs of oxygenated blood, for selected values of $(\text{CO}_2)_b$ and $(\text{BB}^+)_b$, at least two pairs of values for pH_s and P_{CO_2} were obtained, and these used to locate lines of equilibrium state on the incomplete nomogram. The intersection of related lines was then the location

TABLE III
Constants Used in Calculations
Temperature 37°C.

V_c	$(P)_s'$	$(\text{Hb})'$	CO_2 SOLUBILITY FACTOR, f
	<i>g/L. blood</i>	<i>mM/L. blood</i>	<i>mM/L. blood/mm. P_{CO_2}</i>
Plasma	72.0*	0.0	0.0311†
0.2	57.6	4.0	0.0301
0.4	43.2	8.0	0.0292
0.6	28.8	12.0	0.0282
Red cell	0.0	20.0	0.0262‡

$$\text{pK}_s = \text{pH}_s - \log \frac{(\text{CO}_2)_s - 0.0311 P_{\text{CO}_2}}{0.0311 P_{\text{CO}_2}} = 6.11\dagger$$

* From Dill, Edwards and Consolazio, *J. Biol. Chem.*, **118**, 635 (1937).

† From Van Slyke, Hastings, Sendroy and Neill, *J. Biol. Chem.*, **78**, 765 (1928). Values corrected from 38° to 37°C. by adding 3%.

‡ From Dill, Daly and Forbes, *J. Biol. Chem.*, **117**, 569 (1937), and Hastings, Sendroy and Van Slyke, *J. Biol. Chem.*, **79**, 183 (1928). Correction from 38°C. to 37°C. of +0.01.

for the selected value of $(\text{CO}_2)_b$ or $(\text{BB}^+)_b$. From a sufficient number of these points the scale 1 and 3 grids were constructed, with a certain amount of interpolation.

Many calculations were necessary to determine points for the construction of the preliminary graphs. They were accomplished in the manner indicated in the following sample calculation, with the aid of the constants and basic data given in Tables III and IV.

Assume whole blood buffer base $(\text{BB}^+)_b = 50 \text{ mEq/L}$, $V_c = 0.6$, $\text{pH}_s = 7.40$, and O_2 saturation 100%. The first step is to calculate the plasma and red cell protein anion concentrations *per liter of whole blood*.

$$(P^-)_s' = (0.241) (P)_s' = (0.241) (28.8) = 6.9 \text{ mEq/L. blood} \quad \text{eq. 1}$$

$$(P^-)_c' = (2.06) (\text{Hb})_c' = (2.06) (12.0) = 24.7 \text{ mEq/L. blood} \quad \text{eq. 2}$$

$$(P^-)_b' = (P^-)_s' + (P^-)_c' = 6.9 + 24.7 = 31.6 \text{ mEq/L. blood} \quad \text{eq. 3}$$

The buffer base is, by definition, the sum of the "bicarbonate" (including carbamino CO_2) and non- CO_2 buffer anion concentrations:

$$(\text{HCO}_3^-)_b = (\text{BB}^+)_b - (\text{P}^-)_b = 50.0 - 31.6 = 18.4 \text{ mEq/L. blood} \quad \text{eq. 4}$$

The CO_2 pressure is as yet unknown, so $(\text{CO}_2)_b$ cannot be used to obtain $(\text{CO}_2)_s$. As a first step in this approximation, we use F , the ratio $\frac{(\text{CO}_2)_s}{(\text{CO}_2)_b}$, to obtain approx. $(\text{HCO}_3^-)_s$.

$$\text{approx. } (\text{HCO}_3^-)_s = F \cdot (\text{HCO}_3^-)_b = (1.310) (18.4) = 24.1 \text{ mEq/L. plasma} \quad \text{eq. 5}$$

TABLE IV
Buffer* and CO_2 Factors Used in Calculations

pH _s	(P ⁻) _s	OXYGENATED				REDUCED			
		calc. pH _s	(P ⁻) _s	(CO ₂) _s /(CO ₂) _b †		calc. pH _s	(P ⁻) _s	(CO ₂) _s /(CO ₂) _b †	
				Vc = 0.2	Vc = 0.6			Vc = 0.2	Vc = 0.6
	mEq/ gm(P) _s		mEq/ mM(lb)	mM/L. serum mM/L. blood	mM/L. serum mM/L. blood		mEq/ mM(lb)	mM/L. serum mM/L. blood	mM/L. serum mM/L. blood
6.80	0.178	6.76	0.49	1.057	1.195	6.74	0.17	1.048	1.159
7.00	0.200	6.91	1.06	1.063	1.222	6.89	0.66	1.051	1.175
7.20	0.221	7.06	1.58	1.076	1.260	7.04	1.15	1.058	1.203
7.40	0.241	7.20	2.06	1.083	1.310	7.19	1.62	1.067	1.239
7.60	0.262	7.33	2.50	1.099	1.376	7.34	2.06	1.081	1.284
7.80	0.283	7.45	2.91	1.199	1.452	7.49	2.50	1.097	1.338
8.00	0.304	7.58	3.29			7.62	2.93		

* From Dill, Edwards and Consolazio, J. Biol. Chem., 118, 635 (1937). Smoothed curves from equations or graphs.

† From Van Slyke and Sendroy, J. Biol. Chem., 79, 781 (1928).

$$\text{But} \quad \text{pH}_s = 6.11 + \log \frac{(\text{HCO}_3^-)_s}{0.0311 \text{ P}_{\text{CO}_2}} \quad \text{eq. (6)}$$

$$\therefore \text{approx. } \text{P}_{\text{CO}_2} = \frac{\text{approx. } (\text{HCO}_3^-)_s}{(0.0311)(10^{\text{pH}_s - 6.11})} = \frac{24.1}{(0.0311)(10^{7.40 - 6.11})} = 39.7 \text{ mm.} \quad \text{eq. (7)}$$

$$\text{And } (\text{CO}_2)_b = (\text{HCO}_3^-)_b + (0.0282) (\text{P}_{\text{CO}_2}) = 18.4 + 1.12 = 19.5 \text{ mM/L. blood} \quad \text{eq. 8}$$

Now the exact $(\text{CO}_2)_s$ can be found from $(\text{CO}_2)_b$ and F :

$$(\text{CO}_2)_s = F \cdot (\text{CO}_2)_b = (1.310) (19.5) = 25.5 \text{ mM/L. plasma} \quad \text{eq. 9}$$

$$\text{But} \quad \text{pH}_s = 6.11 + \log \frac{(\text{CO}_2)_s - 0.0311 \text{ P}_{\text{CO}_2}}{0.0311 \text{ P}_{\text{CO}_2}} \quad \text{eq. (10)}$$

$$\therefore \text{P}_{\text{CO}_2} = \frac{(\text{CO}_2)_s}{(0.0311)(1 + 10^{\text{pH}_s - \text{pK}})} = \frac{25.5}{(0.0311)(1 + 1.95)} = 40.0 \text{ mm.} \quad \text{eq. (11)}$$

$$(\text{H}_2\text{CO}_3)_s = (0.0311) (40) = 1.24 \text{ mM/L. plasma} \quad \text{eq. 12}$$

$$(\text{HCO}_3^-)_s = (\text{CO}_2)_s - (\text{H}_2\text{CO}_3)_s = 25.5 - 1.24 = 24.3 \text{ mEq/L. plasma} \quad \text{eq. 13}$$

$$\text{exact } (\text{H}_2\text{CO}_3)_b = (0.0282) (40.0) = 1.13 \text{ mM/L. blood} \quad \text{eq. 14}$$

$$\text{exact } (\text{CO}_2)_b = 18.4 + 1.13 = 19.53 \text{ mM/L. blood} \quad \text{eq. 15}$$

Similar calculations were carried out for values of $(\text{BB}^+)_b$ of 70, 50, 30 and 23 mEq/L.; for values of V_c of 0.0, 0.2, 0.4 and 0.6; for values of pH_s of 6.8, 6.9, 7.0, 7.2, 7.4, 7.6, 7.7, and 7.8; and for reduced as well as oxygenated blood. $(\text{BB}^+)_b$ and $(\text{CO}_2)_b$ could then be plotted against P_{CO_2} for different levels of pH_s , V_c and oxygen saturation to obtain the graphs preliminary to the construction of the nomogram.

The derivation of the correction factors, f_{CO_2} , and f_{BB^+} , for incompletely oxygenated blood, on scale 7, was a complicated process. Essentially the derivation consisted of the development of empirical equations from the graphs relating $(\text{CO}_2)_b$ and $(\text{BB}^+)_b$ to P_{CO_2} . These were linear relations of the forms

$$(\text{CO}_2)_b = m_1 P_{\text{CO}_2} \quad \text{eq. 16}$$

$$(\text{BB}^+)_b = m_2 P_{\text{CO}_2} + b \quad \text{eq. 17}$$

where m_1 , m_2 and b are all different functions of pH_s , V_c , and the oxygen unsaturation U . Values of m_1 and m_2 and b were accordingly calculated and analyzed for their relationship with these variables. For a constant pH_s and U the variations with V_c were seen to be linear, so values extrapolated to $V_c = 1$ were calculated. Such values of m_1 and m_2 were found to have a nearly linear relation with 10^{pH_s-7} (or $\frac{1}{(\text{H}^+)_s \times 10^7}$), and from these relations the difference between $U = 1$ and $U = 0$ could be obtained. Similar treatment of b extrapolated to $V_c = 1$ gave a nearly linear relation with pH_s , from which the difference between $U = 0$ and $U = 1$ could likewise be derived. The final equations were these:

$$\text{Reduced } (\text{CO}_2)_b = \text{Oxyg } (\text{CO}_2)_b + [(0.022) (10^{\text{pH}_s-7}) - 0.010] \cdot P_{\text{CO}_2} \cdot U \cdot V_c \quad \text{eq. 18a}$$

$$= \text{Oxyg } (\text{CO}_2)_b + f_{\text{CO}_2} \cdot U \cdot V_c \quad \text{eq. 18b}$$

$$\text{Reduced } (\text{BB}^+)_b = \text{Oxyg } (\text{BB}^+)_b - \{[(0.022) (10^{\text{pH}_s-7}) - 0.010] P_{\text{CO}_2} - 8.5\} \cdot U \cdot V_c \quad \text{eq. 19a}$$

$$= \text{Oxyg } (\text{BB}^+)_b - f_{\text{BB}^+} \cdot U \cdot V_c \quad \text{eq. 19b}$$

There is a theoretical basis for these equations that need not be entered into here. Values of f_{CO_2} and f_{BB^+} are thus readily calculated from pH_s (scale 6) and P_{CO_2} (scale 5) and located on the nomogram as scale 7.

PULMONARY INSUFFICIENCY

I. PHYSIOLOGICAL CLASSIFICATION, CLINICAL METHODS OF ANALYSIS, 897 STANDARD VALUES IN NORMAL SUBJECTS¹

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INTRODUCTION

Pulmonary insufficiency may be defined as a pathological state producing physical disability, and caused by disordered or inadequate functioning of the lungs. This may be primary, due to intrinsic pulmonary disease, or secondary, as when pulmonary function is reduced in various systemic diseases.

About six years ago (1), two of the present authors suggested a simple classification of pulmonary insufficiency, described a series of methods for the analysis of pulmonary functions, and demonstrated the application of these methods in the study of forty-five cases of tuberculosis, before and after thoracoplasty. Since then, these methods have been extended (2, 3, 4, 5, 6, 7, 8) and applied to the analysis of a large number of cases of various types of pulmonary and cardio-pulmonary disease. In the course of this work, our ideas about pulmonary insufficiency have also developed further and have become to some extent better integrated.

In the present paper we shall restate our basic classification of the forms of pulmonary insufficiency, present in detail the methods which we are now using, with our interpretations of their usefulness and limitations, and give, as standards for subsequent studies, the results of measurements, from our own and other laboratories and clinics, in groups of normal subjects.

It is essential at the outset that the scope of the term pulmonary function as we are considering it, be defined, with respect to what it excludes as well as what it includes. Primarily it is the functioning of the lungs as such. For the purpose of the present discussion, the respiratory stimulus will be more or less taken for granted. In this respect, the coverage of the term "pulmonary insufficiency," as we see it, is more restricted than, for example, that of the term "respiratory insufficiency," as employed by Anthony in his excellent monograph on the subject (9). Furthermore, the extent to which the cardiac and circulatory aspects of pulmonary insufficiency are considered here will be very limited, and treated only as they concern the immediate performance of the lungs as organs of ventilation and gas exchange.

Physiologically, and to a certain extent anatomically, pulmonary function can be divided into two broad categories:

(1) *Ventilation*, the mass displacement of air between the outside atmosphere and the interior of the lungs. Normal ventilation requires the integrity of the

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bony framework of the chest and adjacent parts, and of the muscles employed in pulmonary ventilation; normal structure and elasticity of the pleura and mediastinal contents; normally elastic and expansible lungs; patent air passages; and a normal respiratory stimulus, as transmitted from the respiratory center, such stimulus being in turn the summation of all nervous influences reaching the center from the ventilatory apparatus itself and from other parts of the body.

This relatively simple physiological function is thus served by a large number of anatomical units: dynamic (the various respiratory muscles); supportive (thorax, shoulder girdle, spine, abdomen, pleura, mediastinum); passive (lung and cardiovascular tissues); regulatory and "self-cleansing" (respiratory muscles and bronchial, laryngeal, and nasopharyngeal muscles and mucous membranes, and the entire nervous apparatus concerned with ventilatory function).

(2) *Respiratory gas exchange*, the exchange of oxygen and carbon dioxide between the blood in the pulmonary capillaries and the outside air. While dependent also on mass ventilation, respiratory gas exchange is more particularly concerned with the effective distribution of inhaled air to the functioning alveoli ("effective tidal air"), and with diffusion of the respiratory gases across the alveolo-capillary membrane.

This function is thus partly served by that of ventilation, also by the special manner in which air is conveyed along the bronchial passages to the alveoli; but it is ultimately an alveolar function as such, in both its distributive and diffusional aspects. As a convenient approximation, respiratory gas exchange may therefore also be termed alveolo-respiratory function.²

The allocation of the process of distribution of inhaled air, or in other words the process of *intrapulmonary mixing*, to the alveolo-respiratory rather than the ventilatory category, is somewhat arbitrary; but, as later discussion will show, it has been found both useful and logical in the development of the concept of alveolar function.

Ventilatory Insufficiency. Adequacy or inadequacy (insufficiency) of ventilatory function depends for any given metabolic state, on the relation between actual ventilation, and the maximum ventilatory capacity of which the individual is capable.

The actual ventilation is the resultant of many factors: the metabolic state of the organism; the anatomical limitations of the pulmonary or cardiocirculatory apparatus; the intensity of the respiratory stimulus, including the basic chemical stimuli and the modifications, both in amount and form of breathing produced by psychological and other nervous influences. The pulmonary ventilation by a given oxygen consumption is expressed by the *ventilatory quotient*. Usually there will be a relatively constant ratio between the volume of air breathed and the volume of oxygen consumed. In altered conditions, as in disease, this ratio may be altered. The *ventilatory quotient* is a useful index of the adequacy of ventilation.

The maximum breathing capacity, or maximum minute ventilation, is determined by the integrity of the anatomical structures above described, and is a function having well defined limiting values for normal subjects. Correspondingly, a pathological diminution in maximum breathing capacity is caused by abnormality in one or more of the structures involved, actively or passively, in ventilation.

The forms of ventilatory insufficiency encountered clinically can be divided into two groups: those in which ventilatory capacity is decreased because of narrowing or partial obstruction of pulmonary airways; and those in which ventilatory capacity is decreased because of restriction in pulmonary expansion and contraction, from one or another dynamic or structural cause other than obstruction of airways, such as: pulmonary fibrosis or other cause of decreased pulmonary elasticity, pulmonary congestion, pneumothorax, kyphoseoliosis, etc.

As a clinical symptom ventilatory insufficiency becomes apparent chiefly as dyspnea, or distressed breathing. In most forms of pulmonary insufficiency the relation holds that dyspnea is experienced when the individual's *ventilatory capacity* cannot easily provide the *actual ventilation* required.

The factor of hyperventilation, or increased volume of ventilation for a given task, has been stressed in most physiological discussions of the phenomenon of dyspnea (10, 11, 12, 13). Quantitatively, such conditions as unequal distribution of inhaled air (14, 15, 16, 17, 18, 19), arterial anoxia from any cause, acidosis, and many proprioceptive nervous impulses will increase the respiratory stimulus, effecting ventilation that may be two or even three times the normal values.

Of much greater importance in the dyspnea of chronic pulmonary disease, however, is the other of the two factors, namely, the decrease in maximum breathing capacity, which frequently drops to one-third or even one-fifth of the normal values. This, as Peabody pointed out many years ago, and as various clinical investigators have pointed out since, is the chief cause of dyspnea in chronic pulmonary disease. The breathing reserve in such cases, that is, the excess breathing capacity over and above the actual ventilation of the moment, is well correlated with the appearance and extent of dyspnea.

The above relation applies, as has been stated, quite satisfactorily to cases of chronic pulmonary disease. There are other conditions, such as cardiac failure, in which the relation does not hold as well. Patients with congestive heart failure complain of dyspnea at times even when their breathing reserve is well maintained. But it should be noted that there are actually several kinds of dyspnea encountered clinically. That of the patient with chronic pulmonary disease, such as pulmonary fibrosis, or pulmonary emphysema, or kyphoseoliosis, or a post-thoracoplasty case is usually simple breathlessness. There are other patients in this category, who, during exercise, scarcely ventilate at all, so that their distress is suffocation rather than breathlessness. They are dyspneic in post-exercise states. The cardiac patient's distress is even more complex: with his hyperventilation, there is a powerful urge to inspire immediately at the end of expiration, apparently an increased Hering-Breuer reflex. There is also, especially after exercise, a profound muscle weakness and sense of exhaustion. There

may be a sense of oppression which is of cardiac origin. All these factors may be included in the distress which he describes as "shortness of breath."

The methods for the measurement and analysis of ventilatory function are in most respects adequate and are relatively simple.

Defective Respiratory Gas Exchange, or Alveolo-Respiratory Insufficiency. In normal individuals, inhaled air is distributed to functioning (perfused) alveoli with remarkable efficiency. If one accepts 150 cc. as an approximate normal value for the anatomical pulmonary dead space (nasopharynx and non-respiratory airways), then nearly all the remaining air is delivered to functioning alveoli, which are both aerated and perfused (19).

In pulmonary disease, poor distribution of inhaled air may be due to: shallow breathing; underventilation of relatively well perfused alveolar spaces, such as may occur in the dilated alveolar spaces of pulmonary emphysema; overventilation of other lung spaces which have little or no perfusion by pulmonary blood. In the condition in which overventilation of poorly perfused lung spaces predominates, the result is merely an inefficient rate of gas exchange with no change in the respiratory gas contents of the arterial blood. The more common result of poor distribution of inhaled air is that the average unit of blood flows through poorly aerated lung spaces, with lower than normal oxygen tension and higher than normal carbon dioxide tension. This will usually be partially compensated by an increase in total ventilation, the increased respiratory stimulus being provided by increased blood carbon dioxide tension, lowered oxygen tension, or both.

Diffusion of respiratory gases between alveolar air and pulmonary capillaries is anatomically dependent on: the network of pulmonary capillaries lining the alveolar spaces, providing sufficient surface for diffusion; total blood flow through the lungs which is adequate in volume and in speed of flow, for oxygen uptake and carbon dioxide elimination; an alveolo-capillary membrane permitting free diffusion of gases.

Due to the rapid diffusion of carbon dioxide through water solutions, the partial pressures of this gas in arterial blood and in alveolar air are identical. In oxygen tensions there is a small but definite gradient, that of alveolar air being normally 5 to 10 mm. Hg above that of arterial blood under conditions of rest, according to the studies of Lilienthal, Riley, et al. (23a). Conroe and Dripps (23b), however, found the alveolo-capillary oxygen gradient to be on the average only about 1 mm. Hg, although showing considerable variation in individual cases.

Abnormalities in the structures providing for diffusion of respiratory gases lead to inadequacy of this function, which is manifested chiefly by lowered arterial oxygen tension, sometimes also by increased arterial carbon dioxide tension. Often such defects will be added to those of poor intrapulmonary mixing.

There are, of course, other factors, chiefly circulatory, which must be considered in the problem of defective respiratory gas exchange, such as: venous admixture in the arterial blood, produced by return flow from bronchial arteries into pulmonary veins (in minimal amount a normal phenomenon), or by truly abnormal venous shunts; reduced total blood flow; alterations in acid-base equilibrium in the blood.

The chief symptoms of alveolo-respiratory insufficiency are those of anoxia and hyperventilation, as will have been apparent from the above discussion.

The measurement of intrapulmonary mixing of inhaled air, or the distribution factor, with an accuracy sufficient for clinical purposes, is a simple procedure. The ultimate resultant of distributive and diffusional factors, namely, arterial blood oxygen saturation, carbon dioxide tension, and pH , can also be readily measured. In the present study these were the chief measurements used. The diffusional factor was thus essentially that part of the arterial blood abnormalities that could not be accounted for by abnormal air distribution in the lungs.

More recently, methods have been devised which provide an analysis of (a) the distribution factor by following the course of washing out of nitrogen from the lungs during pure oxygen breathing (19, 20) or of hydrogen-mixing between lung air and the gas in a spirometer (21) and (b) the diffusion factor, by measuring alveolar gas tensions indirectly, and simultaneously the gas tensions in the blood by a direct method (22, 23, 24). Discussion of these methods is beyond the scope of the present paper.

Classification of Pulmonary Insufficiency

FORM	TYPE OF DISTURBANCE	CHIEF SYMPTOMS
1. Ventilatory (a) Restrictive (b) Obstructive	Mechanical	Dyspnea
2. Alveolo-respiratory (respiratory gas exchange) (a) Distributive (b) Diffusional	Mechanical and physico-chemical	Anoxia, hyperventilation

This simple classification, which summarizes the above discussion, is essentially that presented in our earlier paper, though with one or two important modifications.

There were also two other categories included in the classification presented in the previous study. It may be of interest to mention these briefly, as the reasons for their omission will clarify our present ideas on the whole field under consideration.

One of these, previously listed as a third form of pulmonary insufficiency, was that of combined ventilatory and alveolo-respiratory failure. This is now omitted simply because almost every clinical case is an example of both these forms of insufficiency. It is uncommon to find a pure ventilatory insufficiency and even rarer to find a pure alveolo-respiratory insufficiency. Each form affects and is affected by the other. Conversely, it may be emphasized again that our classification is not so much for the purpose of separating clinical cases into one or another type, as it is to permit the quantitative analysis of any given case into the forms of failure which are present and the degree or relative importance of each form in that case.

The fourth category in the previous classification was that of combined cardio-pulmonary insufficiency. This is now omitted not because of its unimportance;

it is, in fact, all important. But such a listing was wholly inadequate. Functionally, it is obvious that the pulmonary and circulatory apparatus are one unit, serving to convey the respiratory gases between tissues and outside environment.

What is, in fact, really wanted is a fully integrated physiological classification of cardio-circulo-pulmonary insufficiency, into which all clinical forms of cardiac, circulatory, and pulmonary disease can be fitted. Eventually this must be worked out.

In this present discussion, then, the analysis is to be directed mainly toward pulmonary insufficiency as such, and toward the pulmonary aspects of cardio-pulmonary insufficiency. The performance of the heart and circulation had to be kept in mind at all times, but as the work was actually carried out, methods and measurements were not sufficiently complete, nor was the classification sufficiently comprehensive, to accomplish a completely integrated cardiopulmonary study. Further efforts in this direction are in progress.

DESCRIPTION OF METHODS

The three main steps in studying pulmonary function consist in:

1. The measurement of lung volumes and maximum breathing capacity, using the spirographic method.

2. The measurement of (a) residual air volume by an open circuit method, in which the nitrogen of the lungs is washed out by continuous inhalation of pure oxygen and collected over a period of 7 minutes, and (b) an index of intrapulmonary mixing by the sampling of alveolar air at the end of this period.

3. The measurement of ventilation, of respiratory gas exchange in the lungs, and of the state of respiratory gases in the arterial blood at rest, during a standard type of exercise, and during a 5 minute period of recovery.

Two other tests are described, which have occasionally been used in this series:

4. The separate measurement of vital capacity, ventilation, and respiratory gas exchange in each lung, using the bronchspirometric method.

5. The infusion test, which consists in observing the variations of venous pressure, vital capacity, and circulation time during the administration of a saline infusion of 1500 cc. over a 30 minute period. This test, used routinely early in these studies, has been restricted recently to a few selected cases.

I. Spirographic Measurements of Lung Volumes and Maximum Breathing Capacity

The spirographic method is used to record directly: (a) the lung volume changes during quiet breathing and deep breathing, (b) the maximum breathing capacity, and (c) the form of the respiratory tracing.

A. Measurements. 1. *Lung volumes.* Although the lung volumes have been studied by many observers, it is sometimes difficult to compare their data because of the different points of reference for their measurements and the different terms used in their classification (9). The subdivision of lung volumes used in this series was first proposed by Christie (25) and adopted with a minor change by Hurtado and Boller (26). The resting pulmonary mid-position is the point of reference from which all measurements are taken. It is the position to which the thorax returns at the end of a quiet expiration: the volume of air contained then

in the chest is the sum of the reserve and residual air, (the functional residual air of Lundsgaard (27), Binger (28, 29), Christie (25), and Robinson (30); the mid-capacity of Anthony (9), Hurtado et al (26).

The residual air volume remaining in the chest after a maximum expiration is the only lung volume which cannot be measured by spirometry. All the other lung volume changes measured from the mid-position are classified as follows (fig. 1):

a. The tidal air or the average volume of air moved in and out of the chest during breathing.

b. The complementary air or the maximum volume of air inspired from the resting pulmonary mid-position and including the whole tidal air.

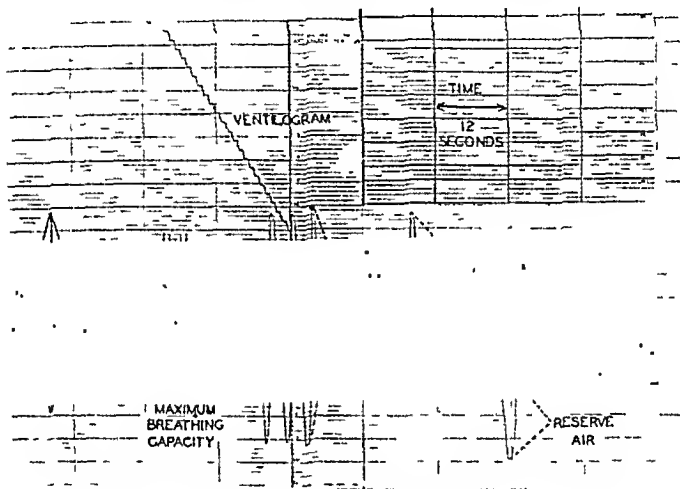


FIG 1. SPIROGRAPHIC RECORDS OF LUNG VOLUMES AND MAXIMUM BREATHING CAPACITY (SEE TEXT FOR DISCUSSION)

c. The reserve air or the maximum volume of air exhaled from the resting pulmonary mid-position.

d. The vital capacity or the maximum volume of air that can be exhaled following a maximum inspiration.

e. The combined vital capacity or the sum of the complementary and reserve air. This is essentially the same as the vital capacity in normal subjects, but may be quite different in abnormal states.

2. *Maximum breathing capacity.* The maximum breathing capacity (31) (maximum minute ventilation or maximum voluntary ventilation) is the largest volume of air that can be moved in and out of the chest in a given period of time by voluntary effort. It is measured in liters per minute.

B. *Apparatus.* The apparatus used is a modified recording spirometer of the

closed circuit type, derived from the Benedict Roth apparatus in general use for basal metabolism determinations. The main modifications from the standard apparatus are the following:

1. The spirometer bell is of 9 liters capacity.
2. The soda lime container and the flutter valves have been removed in order to reduce resistance.
3. Large rubber tubing of one and one half inches inside diameter is used to connect the mouthpiece with the apparatus.
4. A motor-driven kymograph with a speed five times as rapid as the ordinary basal metabolism apparatus of the Benedict Roth type (16 cm. horizontal displacement per minute).
5. A reduction gear is attached to the pulley over which the spirometer compensator chain passes. This reduces the excursion of the pen on the kymograph drum to about one half the excursion of the spirometer bell.³
6. A recording ventilometer is attached to the same pulley. This records the inspiratory excursions of the spirometer only, as well as reducing their amplitude 25 times. The slope of the tracing measures the total ventilation.

C. Procedure. The procedure is thoroughly explained to the subject before he is attached to the apparatus. The spirometer is three quarters filled and its temperature recorded. After the mouthpiece has been placed between the lips and teeth and the nose clip attached, the kymograph is turned on and a few quiet respirations are recorded. The subject is then directed, at the beginning of a quiet expiration to exhale, without an initial inspiration, as much air as possible from his chest, thus recording the reserve air. This is followed immediately by a maximum inspiration and in turn by a maximum expiration. A series of two or three maximum expirations and inspirations is recorded in this manner in order to determine the vital capacity. The subject is then directed to breathe in and out of the spirometer as quickly and deeply as possible for about 15 seconds (see fig. 1). Emphasis is placed upon his attaining the greatest speed possible. It is usually necessary to urge and encourage him to a continually increasing effort in order to obtain the maximum and most satisfactory result. Occasionally in uncooperative subjects or suspected malingerers, the addition of a small amount of carbon dioxide to the oxygen mixture in the spirometer bell will more effectively produce hyperventilation than voluntary effort (32). After a short rest period the spirometer is refilled with room air and the subject connected again with the apparatus. At this time, after a few normal breaths are recorded, he is directed at the end of a quiet expiration to inhale as deeply as possible, in order to measure the complementary air volume; this measurement is then in turn followed by another group of vital capacity and maximum breathing capacity measurements. The tracings of the lung volumes are directly measured in centimeters as shown in fig. 1. These measurements are then converted to cubic centimeters at 37° C. by use of the bell factor and the temperature correction factor. The maximum breathing capacity is similarly calculated in liters per minute at 37° C.

³ In a recent modification of the apparatus the cross section area of the bell has been doubled and the reduction gear therefore eliminated.

D. Discussion of Method. The major source of error in any measurement of the lung volumes and maximum breathing capacity is the failure to obtain the full cooperation of the patient. The measurement of the tidal air, after the first few breaths, is obviously inaccurate due to the building up of carbon dioxide within the system. Since, however, we have other opportunities to measure the tidal air (see section II below) it seems justifiable, for the purpose of reducing the resistance of the system, to remove the soda lime filter from the system, as already described above. With regard to the measurement of maximum breathing capacity, the inertia of the bell and the slight frictional resistance of the recording devices must result in some decrease in the maximum values theoretically obtainable by the use of a frictionless apparatus. These factors may be considered as a more serious error in the measurement of the maximum breathing capacity, involving rapid changes in airflow, than in the measurement of the lung volumes. The greatest error would be expected in subjects with an unusually large maximum breathing capacity (greater than 150 liters per minute) requiring the rapid exchange of large volumes of air in a very short period of time. However, values up to 230 liters per minute have been directly measured. Comparative studies between this method of measurement and another based on the collection of expired air in a Douglas bag, using high velocity valves has in normal subjects failed to reveal any significant discrepancy. Standard values in normal individuals should be, however, determined separately when different types of apparatus are used.

The great advantage of this method of measuring the maximum breathing capacity is the permanent recording of the performance. It is, of course, advisable to be alert to causes of obstruction within the system, such as kinking of the rubber tubing or the sucking of water from the seal into the in- or outflow pipes.

In order to cut down on the resistance still further, we have recently been using rubber tubing with an inside diameter of one and one half inches instead of one inch. A mouthpiece and nose clip are used in preference to a mask because no mask has been developed until very recently which will snugly fit differently shaped faces during forceful breathing without some leaking about the edges.

II. Determination of the Residual Air Volume and of the Index of Intrapulmonary Mixing

The residual air or volume of air contained in the lungs after maximum expiration is measured by the oxygen dilution method of Darling et al (33, 34, 35). The index of intrapulmonary mixing or nitrogen concentration in the alveolar air after a 7 minute period of pure oxygen breathing is determined at the same time.

A. Apparatus. The equipment required is as follows:

1. A special five-way respiratory valve (see fig. 2). By turning the valve (V_1), to which the rubber mouthpiece (M) is attached, the subject is connected to either one of two circuits. When the valve (V_1) is turned into the main circuit, as in the diagram, the subject inhales from a demand valve or a partly filled anesthesia bag of 15 liters capacity, connected with a tank (T) containing

100 per cent oxygen. The exhaled air flows directly through the flutter valve (F_2) into the spirometer (Sp). When valve V_1 is turned to the side circuit the subject inhales and exhales room air from the opening marked room air. For sampling of alveolar air, valve V_2 is turned, as it is in the diagram, to cut off the inspiratory arm of the side circuit. The expiratory arm of this circuit consists of a flutter valve (F_3) and a very short piece of rubber tubing (R) into which is fitted tightly the tip of an evacuated tube for alveolar gas sampling (S_a).

2. A standard 100 liter Tissot gasometer, to the side of which has been attached a long vertical drum that revolves at a constant rate; a pen fastened to the counter weight of the spirometer records each expiratory movement on this drum.

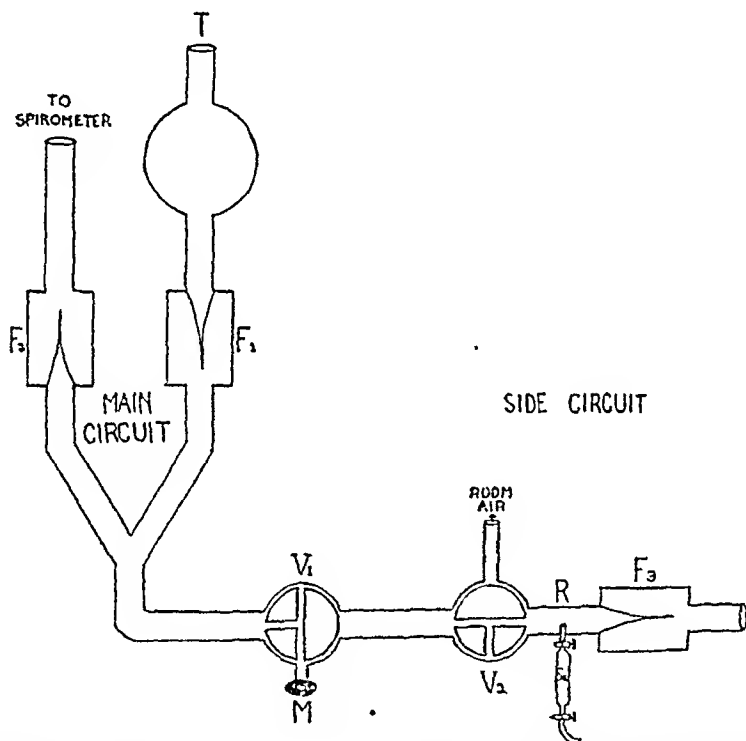


FIG. 2. SCHEMATIC DRAWING SHOWING THE DISPOSITION OF THE TWO CIRCUITS USED IN THE DETERMINATION OF RESIDUAL AIR VOLUME AND THE INDEX OF INTRAPULMONARY MIXING (SEE TEXT FOR DESCRIPTION)

3. Gas sampling tubes.

B. Procedure. The subject should be in a fasting state. Upon his arrival in the laboratory he is made comfortable in the supine position with only a two-pillow head and shoulder elevation. The lung volumes are recorded on the spiograph and the subject is trained to exhale forcibly upon command and to hold his breath in the maximum expiratory position. As soon as the subject exhales relatively constant volumes of reserve air he is given a 15 to 20 minute rest. During this period the main circuit and gasometer are thoroughly washed out with oxygen by six successive fillings and emptyings of the Tissot gasometer, using at least 15 to 20 liters of oxygen for each washing. With the gasometer

empty, volume and temperature readings are taken and oxygen is allowed to flow through the circuit from the tank at about the same rate as the minute ventilation of the subject. An evacuated sampling tube is prepared and attached to the expiratory tubing of the second circuit. The subject is then attached to the mouthpiece with the valve turned to the second circuit so that he is inhaling room air. Precisely at the end of a normal expiration, just before the beginning of inspiration, the respiratory valve V_1 is turned to shift the subject's breathing into the oxygen circuit. The subject then quietly breathes oxygen for a 7 minute period and all the expired air is collected in the gasometer. During this period the second circuit is washed out with oxygen and the inspiratory tubing of the second circuit is shut off by turning the respiratory valve V_2 so that an uncontaminated alveolar sample can be obtained. At the end of an inspiratory phase at the termination of the 7 minute period the valve is turned, and the subject is instructed to exhale completely into the side circuit. An alveolar sample is taken at the very end of the expiration and the subject is immediately disconnected from the apparatus.

The valve is turned at the peak of inspiration in order to provide a larger volume of air for washing out the expiratory arm of the second circuit before alveolar sampling. The amount of nitrogen contained in this tidal air is negligible, so that its loss from the total expired air in the gasometer will cause no error. Finally, the main circuit is flushed out with at least ten liters of oxygen from the tank into the gasometer, so as to wash out any nitrogen remaining in the inflow tubing of the gasometer. The second volume and temperature readings on the gasometer are recorded, and then the inflow as well as the outflow tubing are promptly washed thoroughly with air from the gasometer and two samples are taken from the gasometer for analysis.

A 20 to 30 minute room air breathing period is allowed, providing ample time for the lung nitrogen concentration to return to the preoxygenation figure. Meanwhile, the gasometer and main breathing circuit have again been washed out with oxygen from the tank as described previously. A determination of functional residual air volume is then repeated.

The nitrogen content of the alveolar samples and of the two samples taken from the collected expired air in the gasometer are determined by the use of the VanSlyke-Neill apparatus. The procedure carried out is essentially as described by Peters and VanSlyke (36).

The calculation of the Functional Residual Air is made according to the following formula (10):

$$\text{F.R.A. (dry)} = \frac{(V + DS)(NS - NO) - C}{\text{Alveolar } N\bar{a} - \text{Alveolar } N\bar{p}}$$

where F.R.A. (dry) = functional residual air in cc. calculated as dry gas at standard temperature and barometric pressure.

V = volume of dead space under spirometer in cc. corrected to dry gas, at standard temperature and barometric pressure.

DS = measured volume of dead space under spirometer in cc. corrected to dry gas, at standard temperature and barometric pressure.

NS = per cent nitrogen in spirometer.

NO = per cent nitrogen in oxygen from tank (between 0.0 to 0.5 per cent).

Alv N \bar{p} = per cent nitrogen in alveolar air at end of oxygen breathing.

C = correction for nitrogen excreted from body during period of oxygen breathing, expressed in cc. This factor has been found to vary with bodily size according to the linear relation C in cc. = [(B. S. \times 96.5) + 35] where B. S. is the body surface area in square meters (35).

Alv N \bar{a} = a constant which represents the average nitrogen percentage in the alveolar air at the beginning of the determination and is equal to 81.

The final figure for functional residual air volume is computed from F. R. A. (dry) by correcting for water vapor saturation, as exists (approximately) within the lungs:

$$\text{F.R.A.} = \text{F.R.A. (dry)} \times \frac{\text{Barometer pressure in mm. Hg}}{\text{Barometer pressure in mm. Hg} - 48}$$

The reserve air measured from the spirographic tracing is then subtracted from the functional residual air to obtain the residual air.

C. Discussion of methods. 1. The accuracy of the residual air volume obtained by this method is dependent mainly upon the completeness of washing out, within the 7 minute period of the test, of the lung spaces by the inspired high oxygen. Occasionally nitrogen may not be displaced during the 7 minutes of oxygen breathing from areas of the lung with an intermittent or poor bronchial communication, as in some air cysts of the lung. Hence in such cases the measured residual air volume, i.e., physiological lung volume, will be much lower than the anatomical lung volume calculated on the basis of the radiological measurements (37).

The use of the constant 81 per cent for the nitrogen percentage of the alveolar air at the beginning of the determination rather than a measured value introduces a negligible error. Upon recalculation of data collected from a variety of cases (using the 81 per cent constant for the nitrogen per cent in the alveolar air) an alteration of less than 50 cc. in the functional residual air value was found in 95 per cent of an unselected group of 316 determinations (34).

The most common sources of error in alveolar sampling are: (a) sampling too early during expiration, in which case the air collected in the sampling tube is dead space rather than alveolar air, or (b) waiting too long in a dyspneic subject so that the sample is contaminated by his inhaling room air about the mouth-piece. Therefore, it is wise to gauge the best moment of sampling by observing from the spirographic tracings the average time taken by the subject to deliver his reserve air, as well as his breath holding time. Occasionally a subject may be

encountered with such a restricted reserve air that the volume of alveolar air may not be great enough to wash out adequately the expiratory tubing. In such rare cases the sample of air sucked into the gas sampling tube will be erroneously high in oxygen and worthless.

III. Measurement of Ventilation and Gas Exchange at Rest, during a Standard Exercise Test, and during Recovery

A series of observations are made during a rest period, a standard exercise lasting 1 minute, and the first 5 minutes of the recovery period, consisting in: (a) the ventilation, (b) the respiratory gas exchange in the lungs, (c) the state of respiratory gases in the arterial blood, including oxygen content, capacity and saturation, and carbon dioxide content and tension, and pHs, (d) the pulse rate.

A. Apparatus. The apparatus required includes:

1. A solid platform 20 cm. high, used for the performance of the exercise test.

2. A 100 to 150 liter Tissot gasometer, to which is attached an electrically driven kymograph, with an ink pen attached to the counter weight of the spirometer for recording rate and volume of expiration. (This is the same gasometer used for the residual air determination.)

3. A Douglas bag which is interposed through a threeway valve into the inflow circuit to the spirometer.

4. A system of inspiratory and expiratory flutter valves connected through a T-tube and a mouthpiece to the patient, and through a 2 to 3 foot length of corrugated noncollapsible rubber tubing to the Douglas bag and the Tissot spirometer.

5. Airtight glass syringes, needles, and anti-coagulant solution for blood sampling.

6. Gas sampling tubes.

B. Procedure. The subject, under standard basal conditions, is brought to the laboratory and made comfortable on the bed. Following a 15 to 20 minute rest period, the Douglas bag is evacuated and the respiratory valve turned so as to cut it out of the circuit; the spirometer is washed out twice by 10 to 15 liters of expired air. After a 10 minute rest period, during which time the volume and temperature readings of the empty gasometer are taken, the patient is attached to the mouthpiece and instructed to breathe quietly and easily. The motor of the kymograph is started, the inflow valve to the gasometer opened, the expiratory air collected for a period of 3 to 6 minutes, and the pulse rate determined. At the end of this period the inflow valve is turned off, the patient disconnected from the apparatus and the final volume and temperature readings of the gasometer recorded. Gas samples are taken, and the resting metabolism measurement is thus completed. After another short period of rest the procedure is repeated. The subject is next instructed to stand up in front of the platform, the mouthpiece and nose clip are attached, the flutter valve casings being held by his hand. Upon command he starts to step up on the platform, the right

foot first, followed by the left, and down again in the same order at the rate of 30 such cycles in 1 minute. At the beginning of the exercise period the three-way valve is turned so as to collect the expired air in the Douglas bag. At the end of exactly 1 minute, following the 30th cycle, the subject is directed to lie on the bed without removing the mouthpiece; the three-way valve is turned so that the expired air for the next 5 minutes of recovery is collected in the gasometer, the rate and volume of ventilation being recorded upon the kymograph. In addition, the pulse rate is recorded during each of the 5 minutes of recovery. The patient is repeatedly questioned during the recovery period in an attempt to determine exactly the duration of dyspnea, when it is present. At the end of the 5 minute recovery period, the subject is disconnected from the apparatus.

The ventilation during each minute of rest and recovery is calculated in liters per minute, saturated gas at 37° C. and prevailing barometric pressure, by correcting the corresponding recorded volume by the proper temperature, barometric and bell factors. The average tidal air is then calculated by dividing the total volume of ventilation by the number of respirations. The ventilation, during the period of exercise, is measured by evacuation of the air collected in the Douglas bag into the gasometer and calculating as described above.

Samples of expired air from the three periods of observation are collected in gas sampling tubes from the gasometer and Douglas bag for analysis in duplicate of their carbon dioxide and oxygen content by means of a Haldane apparatus. The air within the Douglas bag is sampled immediately, but that within the gasometer is allowed to stand for at least 5 minutes to allow for proper mixing. The duplicate samples are always taken from two levels of the gasometer. The oxygen consumption and carbon dioxide output are then calculated for the three periods of observation in cc./min. and cc./min./sq. m. B.S. The rate of oxygen removed is calculated as the difference between the inspired and expired air oxygen concentrations, and is expressed in cc./liter of ventilation. In order to account for the excess of inspired air volume over expired air volume, the virtual inspired air oxygen concentration is as follows:

$$\text{Inspired oxygen \%} = 20.93 \times \frac{100 - (\text{Exp. CO}_2 + \text{Exp. O}_2)}{100 - (\text{Insp. CO}_2 + \text{Insp. O}_2)}$$

Arterial blood is obtained at rest and within the first minute of recovery by the puncture of a brachial or femoral artery (36). A careful local anesthesia, using 1 per cent procaine, a sharp short beveled needle and firm pressure at the site of the puncture for 3 to 5 minutes following the withdrawal of the needle makes this a painless, as well as a harmless procedure. An alternative technique developed more recently consists of inserting a specially designed indwelling needle into the brachial artery before the first measurement of ventilation at rest (38). It is then left in place for the remainder of the study, the arm being kept straight with an armboard. This permits repeated withdrawal of blood without pain or difficulty.

The resting arterial blood is obtained after a 20 minute rest period before the basal metabolic determination. Care must be taken especially under conditions

of rest to avoid disturbance of the ventilatory rhythm. During most of the studies to be reported below, the blood was withdrawn and transferred under oil to chilled bottles containing a mixture of dried sodium fluoride and neutral potassium oxalate (39). More recently, the blood has been collected using a more efficient and simpler technique described by Riley (38). Dry syringes are prepared by introducing a small globule of mercury and six drops of heparin and fluoride solution prepared as follows: 600 mg. of sodium fluoride, and 100 mg. of dried heparin in 15 cc. of 0.85 per cent sodium chloride solution. The sides of the syringe are moistened with this solution and the excess expelled through the aperture of the syringe. The amount of solution left is too small to dilute significantly the blood sample. Care is taken so that the drop of mercury used to mix the blood after sampling remains in the space between the rim of the plunger and the barrel. Following the sampling, the needle is disconnected from the tip of the syringe, the opening of which is immediately occluded by a toothpick before it is gently shaken; the mercury serving to keep the sample thoroughly mixed. Blood is transferred under pressure from the syringe to 1 ml. Ostwald-VanSlyke pipettes by way of a small (#25) gauge needle inserted into the tip of the pipette. A small piece of rubber pierced by the needle makes possible an airtight seal. The carbon dioxide and oxygen contents and oxygen capacity determinations are done in duplicate on a VanSlyke-Neill apparatus. In the earlier studies the carbon dioxide tension was read from the carbon dioxide dissociation curve determined with the patient's own blood (39), and the pHs calculated from the line charts of VanSlyke and Sendroy (40). More recently the pHs of the whole blood has been directly measured by means of a modified form of the MacInnes and Belcher glass electrode which permits transfer of blood from a pipette without contact with air. The $p\text{CO}_2$ is then calculated from the line charts of VanSlyke and Sendroy.

C. Discussion of the method. This test can be called standard in that each subject performs a standard number of steps within 1 minute of time. In contrast to bicycle exercise the task is proportional to the size of the individual. In this respect the test resembles that of treadmill exercise; but compared to treadmill exercise it has the following disadvantages: (a) the effort expended during its performance will vary according to the subject's degree of cooperation, (b) the duration of the exercise is far too short to permit the development of a steady state, (c) likewise the duration of the recovery period is too short for the measurement of the oxygen debt, and (d) finally the exercise itself is so mild that it taxes only the obviously handicapped subject, and therefore cannot be expected to demonstrate incipient cardiopulmonary disability. On the other hand, the mild and accustomed nature of the exercise, its short duration, and the ease of performance makes it a suitable test for the hospitalized or severely compromised subjects, who rarely are unable to complete it. It has, finally, the advantage of simplicity of equipment and of execution. It differs from the step tests of Nylin (41) and Master (42, 43) mainly in that (a) the measurements are taken during the exercise as well as during the recovery period and (b) the data obtained are more comprehensive. Furthermore, the exact determination

of the oxygen consumption from the analysis of the expired air, rather than from the slope of spirographic tracings eliminates errors due to changes of respiratory mid-position and arterial anoxia, which are not unusually encountered in subjects with chronic pulmonary disease.

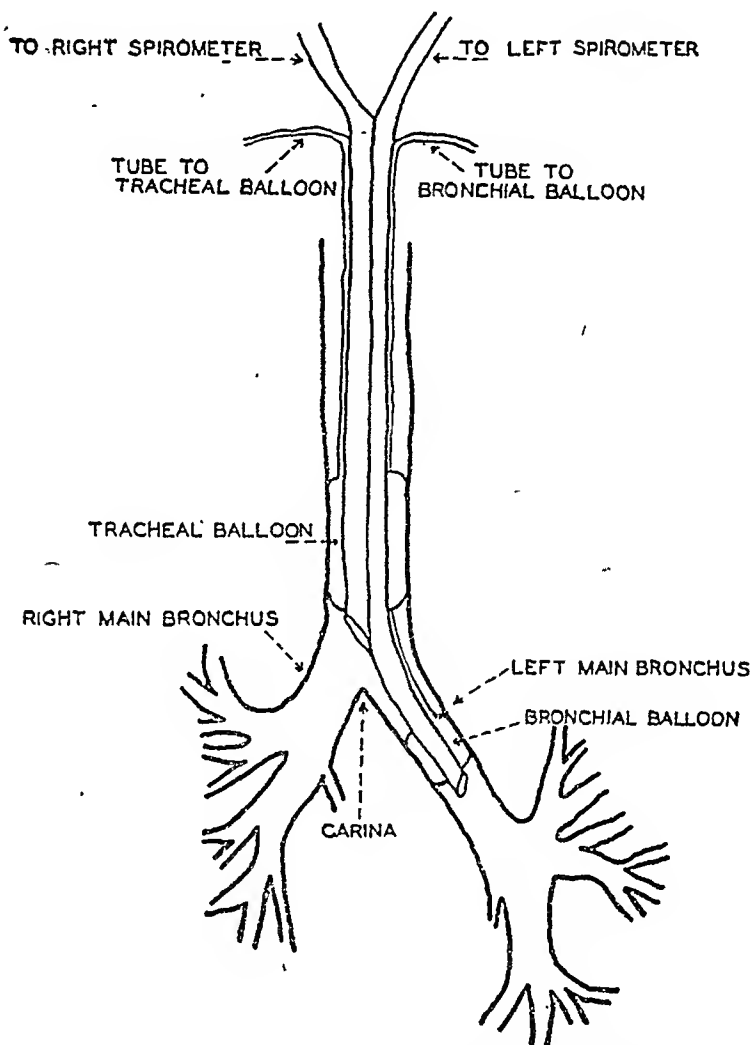


FIG. 3. SCHEMATIC DRAWING ILLUSTRATING THE FLEXIBLE DOUBLE AIRWAY RUBBER TUBE USED FOR BRONCHOSPIROMETRY (SEE TEXT FOR DESCRIPTION)

IV. Bronchospirometry

This method devised by Jacobaeus, Frenckner, and Bjoerkman (44) for the separate measurement of ventilation, gas exchange, and vital capacity of each lung has been used in the studies of a few cases in this series.

A. Apparatus. 1. A flexible double air-way tube made of rubber, a schematic representation of which can be seen in figure 3. The substitution of this tube by Gebauer and by Zavod (45) for the originally used rigid metallic double air-way bronchoscope has introduced a great simplification in this method.

2. Two standard type basal metabolism apparatus for measuring the vital capacity, the ventilation, and the oxygen intake of each lung separately.

3. A double open circuit system with two collecting bags, proper valve arrangements, and small dead space may be used in place of the basal metabolism apparatus for measurement of ventilation, oxygen intake, and carbon dioxide output.

B. Procedure. A detailed description of the procedure used for the introduction of the tube and of the equipment employed for the graphic recording of lung volumes, ventilation, and oxygen intake may be found elsewhere (45, 46, 47, 48). Some emphasis should be given to the following details: (a) Before introduction of the tube, the subject should receive adequate doses of sedatives and atropine. (b) Local anesthesia of the pharynx and larynx should be perfect, and obtained preferably by application of a 5 per cent cocaine and 1 per cent pontocaine solution, freshly prepared, with a special brush rather than by spraying, in order to avoid the unnecessary swallowing of anesthetic solution. (c) A few cc. of solution, in fractionated doses, should be introduced through the larynx after the gag reflex is no more present, and by proper position made to run into the trachea and left main bronchus. The tip of the tube, properly curved, may be introduced through the larynx without any guide while the patient holds his tongue out as far as possible.

C. Discussion of method. The most important step in obtaining reliable results is to place the tip between the trachea and the opening of the left upper lobe bronchus in such a fashion that, once the cuff near the tip is inflated, it neither obstructs the upper bronchus nor impinges upon the tracheal carina. A sudden change in the patient's respiration, the disappearance of breath sounds over the area of the upper lobe bronchus, or the development of a localized wheeze are indications of obstruction of a major bronchus and of improper placement of the tube. After inflation of both cuffs, a recording of vital capacity and ventilation from each lung, using two separate basal metabolism apparatuses, is required in order to ascertain that the tube is well placed, and that there is no leak in the system, especially no abnormal communication between the right and left main bronchi, which are assumed to be separated. Obstruction of a major bronchus is shown by the demonstration of an unusually large difference between the vital capacity measured before bronchospirometry and the sum of the vital capacity of each lung measured separately. It is suspected when the contribution of each lung in the measurement of vital capacity and ventilation deviates too much from the rough estimation made before bronchospirometry on the basis of a careful fluoroscopic study. Abnormal communication between the two separate main bronchi is obvious when one of the ventilatory tracings shows a sharp upward slope, and the other has a downward slope. It is, however, sometimes quite difficult to decide, in the presence of a ventilatory tracing, the slope of which is neither downward nor upward but nearly horizontal, whether the finding is genuine. It can be said in general that whereas a good anesthesia is only a question of care, the reliability of the test, depending upon the proper location of the inflated cuffs, for the separation of both right and left

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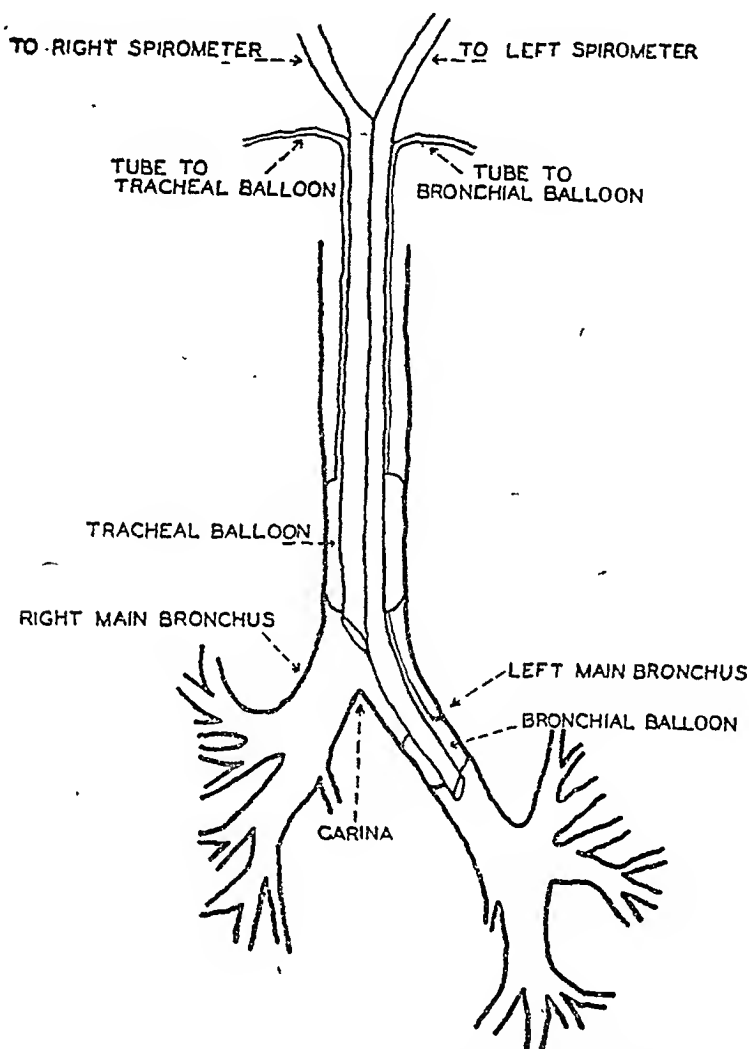


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air-ways, with maintenance of their patency, is dependent upon the mastery of small details that can only be acquired by experience.

V. *The Caughey Infusion Test*

This test consists in the measurement of the venous pressure, the vital capacity, the pulse rate, the arterial blood pressure, and the circulation time before, during, and after the intravenous infusion of 1500 cc. of saline in 30 minutes time (49, 50).

A. *Apparatus*. 1. A standard infusion set including a large gauge (#17) needle and three-way stopcock.

2. A venous pressure apparatus of the water manometer type.

3. The spirometer described above or a standard basal metabolism machine.

4. Calcium gluconate, decholin, or sodium cyanide for circulation time determination.

B. *Procedure*. The subject is made comfortable in the recumbent position. After a short period of rest the venous pressure apparatus is adjusted so that the zero point corresponds to 5 cm. below the angle of Louis (51). The vital capacity, blood pressure, and pulse are measured. The needle is then inserted into a convenient antecubital vein and the venous pressure measured through the side arm of the three-way stopcock. As soon as the venous pressure becomes constant, the circulation time, arm to tongue, or arm to carotid sinus is obtained and the intravenous infusion is started at a rate of about 50 cc. per minute. For the next 30 minutes the venous pressure and pulse are followed about every 3 minutes and the vital capacity is measured once or twice. It is obvious that caution must be observed in performing this test upon any patient with diminished cardiac reserve. If there should be a significant rise in the venous pressure, drop in the vital capacity, or development of cough the infusion is immediately stopped. Otherwise the full 1500 cc. is run in by the end of 30 minutes, at which time the circulation time is repeated, the venous pressure observed for about 10 minutes and a final vital capacity recorded.

MEASUREMENTS IN NORMAL SUBJECTS AND INTERPRETATION OF RESULTS

Data have been assembled in 3 groups of "normal" subjects using the tests described in this paper and statistics have been calculated.

A. In a first group consisting of 52 males and 40 females known to be free clinically from pulmonary and cardiocirculatory disease the following measurements were carried out: lung volumes with the exception of residual air, maximum breathing capacity, and ventilation and pulmonary gas exchange at rest, during the test exercise, and during recovery. The statistics were calculated for males and females separately and are presented in tables 1 and 2, arranged in 3 columns according to age: (1) from 16 to 34 years, (2) from 35 to 49 years, and (3) from 50 years on. Formulae for the prediction of vital capacity and maximum breathing capacity were calculated on the basis of the data collected in this group (table 3). For the prediction of the total capacity use was made of figures for

the ratio $\frac{\text{Vital Capacity}}{\text{Total Capacity}} \times 100$ calculated by Kaltreider (52) on the basis of a

TABLE 1

Measurements in a Control Group of 52 Mole Hospital Patients without Clinical or X-ray Evidence of Pulmonary or Cardiac Disease Arranged in Groups According to Age

	GROUP I: AGE 16-34 YEARS				GROUP II: AGE 35-49 YEARS				GROUP III: AGE 50-69 YEARS			
	No.	Mean	S.D.	Range	No.	Mean	S.D.	Range	No.	Mean	S.D.	Range
A. Physical characteristics												
Age years.....	18	25.5	±6.0	10-34	15	42.7	±4.12	36-48	19	59.6	±5.4	50-69
Height cm.....	18	173.8	±8.6	150-182	15	171.7	±6.93	154-182	19	169.6	±8.3	154-183
Weight kg.....	18	66.0	±8.3	52.5-86.0	15	64.9	±11.2	49-86	19	66.3	±8.6	50-81
B. S. area sq. M.....	18	1.77	±0.14	1.55-2.07	15	1.80	±0.16	1.45-2.00	19	1.80	±0.15	1.51-2.02
B. Vital capacity supine in cc....	17	4012	±616	2792-4950	15	4160	±480	3300-5240	18	3417	±825	2184-5429
C. Standing maximum breathing capacity L/min.....	17	126.0	±23.6	82-169	15	109.4	±15.9	86-144.5	18	90.6	±16.8	58-139
D. Ventilation L/min./sq. M B. S.												
Basal.....	17	3.6	±0.3	3.1-4.5	15	3.1	±0.5	2.6-4.0	19	3.9	±0.45	3.2-4.9
1 min. standard exercise....	17	11.0	±2.3	6.6-17.0	15	10.0	±2.3	5.3-14.2	19	11.2	±2.7	7.4-18.6
1st. min. recovery.....	17	12.5	±2.1	9.2-16.1	15	13.4	±2.6	10.2-18.9	18	14.5	±2.5	10.1-20.3
2nd. min. recovery.....	17	8.6	±1.5	6.3-11.8	15	9.4	±1.9	6.5-13.5	18	10.8	±2.0	5.7-14.4
5th. min. recovery.....	15	5.2	±0.63	2.8-7.0	15	5.2	±0.7	4.2-6.7	18	6.3	±1.2	4.1-8.6
E. Oxygen consumption cc/min./sq. M B. S.												
Basal.....	17	146	±14	129-186	15	131	±10	118-156	19	132	±17	107-165
1 min. standard exercise....	17	503	±80	400-739	15	481	±90	276-578	19	506	±90	283-757
5 min. recovery.....	16	1488	±172	1114-1735	15	1493	±96	1360-1662	18	1511	±164	1308-2034
F. Oxygen removal cc/L ventil.												
Basal.....	17	47.1	±5.0	36.3-53.8	15	46.1	±5.5	37.4-53.8	19	38.5	±2.4	34.3-44.7
1 min. standard exercise....	18	56.2	±7.4	43.7-73.2	15	55.7	±6.7	44.6-63.0	19	53.2	±6.7	40.3-64.1

TABLE 2

Measurements in a Control Group of 40 Female Hospital Patients without Clinical or X-ray Evidence of Pulmonary or Cardiac Disease Arranged in Groups According to Age

	GROUP I: AGE 16-34 YEARS				GROUP II: AGE 35-49 YEARS				GROUP III: AGE 50-79 YEARS			
	No.	Mean	S.D.	Range	No.	Mean	S.D.	Range	No.	Mean	S.D.	Range
A. Physical Characteristics												
Age years.....	17	25.1	±6.2	15-34	10	43.3	±3.6	36-47	13	59.8	±9.0	50-79
Height cm.....	17	161.8	±6.2	154-172	10	164.0	±6.8	148.5-172	13	158.4	±6.7	147-167
Weight kg.....	17	59.2	±11.1	44.0-84.0	10	62.6	±15.3	51.0-104.5	13	67.2	±11.0	53.5-93.0
B.S. area sq. M.....	17	1.58	±0.14	1.39-1.94	10	1.72	±0.17	1.46-2.10	13	1.7	±0.12	1.46-1.87
B. Vital capacity supine in cc....												
	16	3057	±551	2312-4150	10	2830	±397	2212-3435	13	2431	±532	1570-3525
C. Standing maximum breathing capacity L/min.....												
	15	93.7	±12.6	63.6-117.5	10	89.3	±17.9	47-114	13	73.5	±16.8	49-101.5
D. Ventilation L/min./sq. M B.S.												
Basal.....	17	3.2	±0.4	2.55-4.27	10	3.2	±0.4	2.40-3.71	13	3.4	±0.40	2.53-3.95
1 min. standard exercise....	17	8.99	±1.7	6.2-12.4	10	11.4	±2.8	7.16-17.5	13	11.4	±1.7	8.70-15.02
1st. min. recovery.....	16	10.9	±1.5	8.0-13.0	10	11.9	±2.7	7.5-15.5	12	12.6	±2.2	8.0-15.6
2nd. min. recovery.....	14	8.1	±1.3	6.5-10.8	10	9.2	±1.7	6.0-12.5	12	8.5	±0.9	6.0-11.4
5th. min. recovery.....	14	4.9	±0.6	3.5-5.5	10	5.2	±1.0	3.0-6.5	12	4.5	±1.1	2.9-6.4
E. Oxygen consumption cc/min./sq. M B.S.												
Basal.....	17	126	±10	111-149	10	126	±9	109-136	13	129	±11	105-150
1 min. standard exercise....	17	463	±76	313-616	10	505	±91	370-676	13	512	±71	366-719
5 min. recovery.....	10	1318	±80	1172-1476	10	1368	±92	1143-1500	13	1348	±135	1170-1648
F. Oxygen removal cc/L ventil.												
Basal.....	17	45.1	±4.3	36.8-52.6	10	46.0	±6.4	38.6-62.3	13	44.5	±3.9	37.2-52.6
1 min. standard exercise....	17	60.2	±9.5	49.7-79.9	10	53.6	±7.4	44.9-68.0	13	53.5	±6.4	41.7-64.0

study of 150 normal male and female subjects. In this ratio the total capacity was equal to the sum of separately measured vital capacity and residual air. Although the Christie method used in Kaltreider's series for the measurement of residual air is different from the method used in our studies, application of his ratios to our figures of vital capacity would seem valid, in view of the demonstra-

TABLE 3
Formulae for the Prediction of Lung Volumes and Maximum Breathing Capacity

	GROUP I, AGE 16-34 YEARS	GROUP II, AGE 35-49 YEARS	GROUP III, AGE 50-69 YEARS
1. Total capacity (supine) Males and females.....	$\frac{\text{Vital capacity}}{60.0} \times 100$	$\frac{\text{Vital capacity}}{76.6} \times 100$	$\frac{\text{Vital capacity}}{69.2} \times 100$
2. Ratio $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$ (supine) Males and females.....	20.0	23.4	30.8
3. Vital capacity (supine), in cc. Males..... Females.....	$[27.63 - (0.112 \times \text{age in yrs.})] \times \text{height in cm.}$ $[21.78 - (0.101 \times \text{age in yrs.})] \times \text{height in cm.}$		
4. Maximum breathing capacity (standing), lit./min. Males..... Females.....	$[56.5 - (0.522 \times \text{age in yrs.})] \times \text{m}^3 \text{ B.S.}$ $[71.3 - (0.474 \times \text{age in yrs.})] \times \text{m}^3 \text{ B.S.}$		

TABLE 4
Arterial Blood Studies in a Control Group of 15 Hospital Patients without Pulmonary or Cardio-circulatory Disease

	MEAN	S.D.
1. <i>At rest</i>		
Oxygen saturation (per cent).....	96.2	± 1.2
Carbon dioxide content (volumes per cent).....	52.0	± 2.4
Carbon dioxide tension (mm. Hg).....	43.7	± 3.5
Carbon dioxide content at 40 mm. Hg (volumes per cent)...	50.6	± 2.3
pH _a at 38° C.....	7.43	± 0.02
2. <i>During first minute of recovery</i>		
Oxygen saturation (per cent).....	95.8	± 1.7
Carbon dioxide content (volumes per cent).....	47.8	± 2.3
Carbon dioxide tension (mm. Hg).....	43.0	± 2.4
Carbon dioxide content at 40 mm. Hg (volumes per cent)...	46.6	± 1.9
pH _a at 38° C.....	7.40	± 0.03

tion previously made that in normal subjects both methods of residual air determination give approximately comparable results (33).

B. In 15 additional normal subjects, the state of the respiratory gases in the arterial blood was studied at rest and following the test exercise. The statistics are presented in table 4.

C. In a group of 39 hospitalized subjects without pulmonary or cardio-circulatory disease (Caughey, unpublished data) the effects of the infusion test were studied. The statistics were calculated and appear in table 5.

The tabulated data and the formulae for prediction of normal values will be discussed for each set of measurements, and compared to similar data to be found in the literature. The basis for a physiological interpretation of any significant departure from normal values will also be considered.

I. Lung Volumes

A. *Vital capacity.* It has long been known that the vital capacity is correlated to a variable degree with a number of physical characteristics. The results of such a study, in both groups of males and females, are presented in table 6. The decrease of the vital capacity with increasing age, its increase with increasing height and its lack of correlation with weight confirm the observations made by others (9, 30, 52). A positive correlation between the vital

TABLE 5

Infusion Test Data in a Control Group of 39 Hospital Patients without Pulmonary or Cardio-circulatory Disease

	MEAN	S.D.
1. <i>Venous Pressure</i> (mm. Hg)		
Before infusion.....	69.4	± 23.0
After infusion.....	78.0	± 37.0
2. <i>Circulation Time</i> (in seconds)		
Before infusion.....	14.5	± 4.0
After infusion.....	13.8	± 3.2
3. <i>Vital Capacity</i> (per cent decrease after infusion).....	4.7	± 3.8

capacity and the body surface area confirms West's early work (53). In table 7 the mean values and range of vital capacity and reserve air measurements made in our group of male subjects are compared according to age with the data published separately by Kaltreider et al (52) and by Robinson (30) in groups of male subjects of similar ages. On the whole the mean values for the vital capacity in our group tend to be considerably lower. However, a comparison of the physical characteristics of the various male groups indicates that on the average the male subjects of our group were at comparable age, shorter and lighter than the male subjects in Kaltreider and in Robinson series. This factor probably explains the discrepancy. In contrast it can be seen from table 8 that in Kaltreider's as well as in our own young female group the physical characteristics were similar, and that the mean figures for vital capacity compared remarkably well.

Since all observers agree that the vital capacity decreases progressively and significantly with age, any formulae for its prediction should be based upon age,

as well as sex and height. The regression formulae in table 3 were therefore calculated with all variables. In spite of the large standard error, respectively ± 0.038 and ± 0.028 in the male and the female groups, these formulae appear as an improvement over those based upon height alone, especially in the older age groups. Because they are based upon observations made on a relatively short and light group of hospital patients, the vital capacities of healthy young sub-

TABLE 6

Various Correlations between (a) Vital Capacity and Maximum Breathing Capacity and (b) Physical Characteristics in a Group of 62 Male and a Group of 40 Female Subjects

	MALES			FEMALES		
	R	Z	P	R	Z	P
Correlation of Standing Vital Capacity with						
Age.....	-0.432	-0.460	0.002	-0.589	-0.670	<0.0004
Height.....	+0.485	+0.526	0.0004	+0.501	+0.550	0.002
Weight.....	+0.227	+0.230	>0.05	+0.067	—	—
Body Surface.....	+0.436	+0.470	0.002	+0.263	+0.265	>0.05
Correlation of Supine Vital Capacity per centimeter of height with						
Age.....	-0.428	-0.455	0.003	-0.505	-0.56	0.05
Correlation of Supine Vital Capacity per square meter of body surface with						
Age.....	-0.408	-0.42	0.005	-0.641	-0.76	<0.0004
Correlation of Standing Maximum Breathing Capacity with						
Age.....	-0.633	-0.720	<0.0004	-0.535	-0.600	0.0004
Height.....	+0.407	+0.427	0.005	+0.460	+0.49	0.002
Weight.....	+0.267	+0.277	>0.05	+0.089	—	—
Body Surface.....	+0.361	+0.381	0.01	+0.238	—	>0.05
Vital Capacity.....	+0.777	+1.05	<0.0004	+0.818	+1.150	<0.0004
Correlation of Standing Maximum Breathing Capacity per sq.m. of B.S. with						
Age.....	-0.627	-0.740	<0.0004	-0.652	-0.780	<0.0004
Correlation of Standing Maximum Breathing Capacity per cm. of height with						
Age.....				-0.513	-0.570	0.001

jects in good muscular training predicted by these formulae might well be erroneously low. The formulae were, therefore, tested by calculating the predicted vital capacities for the various groups of Kaltreider and Robinson, which consisted largely of healthy subjects in good training. As may be seen in table 9, the observed values are somewhat larger than the predicted values but the differences were well below 20 per cent in all 10 groups and below 10 per cent in 8

TABLE 7

A Comparison of Measurements of Lung Volumes in Various Groups of "Normal" Male Subjects

AGE GROUP	AGE IN YEARS	AUTHORS	NO. OF CASES	VITAL CAPACITY			% TOT. CAP.	RESERVE AIR			% VIT. CAP.
				Mean	S.D.	Range		Mean	S.D.	Range	
1	15-35 mean 25.5	Richards, et al.	17	liters 4.01	liters ± 0.62	liters 2.79-4.95		liters 0.88	liters ± 0.20	liters 0.52-1.46	20.6
	18-30 mean 23.0	Kaltreider, et al.	50	4.78	± 0.59	3.40-5.85	80.2	0.98	± 0.26	0.26-1.58	20.6
	16-19 mean 17.5	Robinson	12	4.95		4.12-5.98	70.1	1.18		0.89-1.54	24.0
	20-29 mean 24.0	Robinson	11	5.25		4.20-6.03	76.3	1.39		0.95-1.83	26.4
2	35-49 mean 43.3	Richards, et al.	15	4.29	± 0.54	3.42-5.30		0.88	± 0.36	0.41-1.60	21.4
	38-50 mean 35.1	Kaltreider, et al.	34	4.12			76.6	0.70			16.7
	31-38 mean 35.1	Robinson	11	4.76		3.83-6.49	74.8	0.98		0.37-1.87	20.6
	40-48 mean 44.3	Robinson	10	4.28		3.76-5.16	74.9	0.69		0.23-1.20	16.1
3	50-60 mean 55.8	Richards, et al.	12	3.65	± 0.93	2.21-5.43		0.73	± 0.27	0.36-1.25	20.0
	50-60 mean 51.5	Kaltreider, et al.	10	4.18			74.5	0.84			20.3
	48-55 mean 51.5	Robinson	8	4.16		3.60-5.52	71.4	0.83		0.00-1.61	20.0
	60-69 mean 65.4	Richards, et al.	7	3.27	± 0.60	2.42-4.07		0.72	± 0.27	0.30-0.99	22.0
	60-76 mean 62.0	Kaltreider, et al.	8	3.43			68.5	0.46			14.0
	59-66 mean 62.0	Robinson	7	4.05		3.45-5.04	70.2	0.68		0.38-1.19	16.8
	71-91 mean 76.8	Robinson	5	3.20		2.61-3.40	62.2	0.47		0.18-0.87	14.7

TABLE 8

A Comparison of Measurements of Lung Volumes in Various Groups of "Normal" Female Subjects

AGE GROUP	AGE IN YEARS	AUTHORS	NO. OF CASES	VITAL CAPACITY			% TOT. CAP.	RESERVE AIR			% VIT. CAP.
				Mean	S.D.	Range		Mean	S.D.	Range	
1	16-35 mean 25.1	Richards, et al.	17	liters 3.05	liters ± 0.55	liters 2.31-4.15		liters 0.64	liters ± 0.02	liters 0.18-1.05	20.4
	18-34 mean 23.1	Kaltreider, et al.	50	3.14	± 0.41	2.28-3.95	74.1	0.73	± 0.19	0.28-1.42	22.9
2	35-50	Richards, et al.	10	2.83	± 0.38	2.21-3.44		0.49	± 0.10	0.28-0.78	17.0
3	50-60	Richards, et al.	8	2.70	± 0.53	2.20-3.52		0.35	± 0.18	0.22-0.66	13.0
	60-79	Richards, et al.	5	2.43		1.57-2.72		0.17		0.05-0.45	14.4

of the groups. Since our observations indicate that the vital capacity in the female group decreased with age to about the same degree as that of the male group, it would seem justified, in the absence of data in the literature to be com-

pared with predicted values, to assume that the proposed formula would probably hold true.

In addition of the lung volumes themselves, analysis of the pattern of the respiratory tracing from which the volumes are measured gives valuable information. In normal individuals the rapid and unimpeded flow of air in and out of the chest is demonstrated by the almost perpendicular slope of the inspira-

TABLE 9

Comparison between Observed Values and Predicted Values of Lung Volumes Measured in Various Groups of Normal Subjects

AUTHOR	GROUP	MEAN AGE	MEAN HEIGHT	MEAN VITAL CAPACITY				MEAN TOTAL CAPACITY				ORIGIN OF THE GROUPS
				Measured	Calculated*	Difference		Measured	Calculated*	Difference		
						cc.	per cent			cc.	per cent	
Kaltreider, et al.	50 males	22.9	176.2	4780	4420	-360	7.5	5970	5525	-445	7.5	Medical students
	15-30 yrs.											
	50 males	48.2	170.5	4070	3600	-270	7.1	5370	5075	-295	5.5	Hospital "normals"
	35-63 yrs.											
	50 females	23.1	163.0	3140	3170	+30	0.9	4240	4230	-10	0.2	Nurses
Robinson	18-24 yrs.											
	12 males	17.5	170.0	4950	4600	-350	7.1	6250	5700	-450	7.8	School and college students
	16-19 yrs.											
	11 males	24.0	179.6	5250	4490	-760	15.9	6810	5620	-1190	17.5	College students
	20-29 yrs.											
	11 males	35.0	175.3	4760	4150	-610	12.8	6360	5460	-900	14.2	Faculty members
	31-38 yrs.											
	10 males	44.8	176.6	4290	3960	-330	7.5	5770	5250	-490	8.5	Faculty members
	40-48 yrs.											
	8 males	51.5	172.4	4160	3753	-405	9.7	6310	5230	-1080	17.1	Faculty members
	48-55 yrs.											
	7 males	62.0	177.8	4050	3600	-300	9.6	5770	5300	-470	8.1	Faculty members
	59-66 yrs.											
	5 males	76.8	171.3	3200	2900	-300	9.4	5120	4200	-920	18.0	Faculty members
	71-91 yrs.											

* Formulas for Prediction were:

Vital Capacity in Males cc. = $[27.63 - (0.112 \times \text{age})] \times \text{Ht. cm.}$

Vital Capacity in Females cc. = $[21.78 - (0.101 \times \text{age})] \times \text{Ht. cm.}$

Total Capacity

Between 15-34 years of age = $\frac{\text{vital capacity}}{80.0} \times 100$

Between 35-49 years of age = $\frac{\text{vital capacity}}{76.6} \times 100$

Above 50 years of age = $\frac{\text{vital capacity}}{69.2} \times 100$

tory and expiratory tracing, save for a small portion at maximum inspiration and expiration where the flow is reduced progressively. In addition the tracings return as a rule to the same resting position on successive performances. When restriction in the lung volume is due to ineffective and uncoordinated respiratory chest movements, loss of elasticity of the lung or partial obstruction to the movement of air in any phase of respiration, it will result in slowing of the flow of air

this figure was exceeded in only one determination (34). A nitrogen percentage greater than 2.5 may therefore definitely be considered abnormal and indicative of inadequate ventilation of some areas of lung. It must be recognized, that in hyperpneic subjects, defects of intrapulmonary mixing may well be missed at the end of the 7 minute period.

IV. Ventilation and Breathing Reserve at Rest, during and after the Standard Exercise

As seen in table 1 and table 2, the mean figures of ventilation in the male and female groups, arranged according to age, were remarkably constant for a given state of physical activity, but the range of variation of the observations was wide. There was a slightly greater tendency for the ventilation to be maintained at the higher level in the older age groups than in the younger during the early period of recovery following exercise.

Calculation of the breathing reserve at rest in both male and female groups shows that it varies between 95 and 91 per cent of the maximum breathing capacity up to the fifth decade. In the older age group one occasionally encounters a breathing reserve at rest of 90 to 88 per cent of the maximum breathing capacity. During the first minute of recovery it is more variable, ranging from 88 to 70 per cent in the younger and between 75 to 45 per cent in the older age groups.

Hyperventilation during any or all of the periods of observations is the most common pathological finding. It may be due to reflex, chemical or central nervous system stimulation, acting upon the respiratory centers: (a) Abnormal reflex stimulation may originate in the lungs, (stretch reflexes of the Hering-Breuer type), in the wall of the large veins, right auricle and ventricle (mediated through pressor-sensitive neuro-muscular elements), in the muscles or joints of the chest bellows, or in the muscles and joints of the limbs. (b) Abnormal chemical stimulation may result from increased $p\text{CO}_2$ or a state of acidosis in the arterial blood affecting directly the main respiratory center, or a state of anoxia in the arterial blood affecting indirectly the main center by stimulation of the carotid body. (c) Abnormal stimulation from the higher centers are related to emotional factors or organic lesions.

Occasionally the ventilation is greatly reduced during the performance of the exercise test, in normal subjects. These cases must be distinguished from others who are unable to ventilate properly because of fixation of the respiratory muscles during the performance of exercise (4). In both instances the ventilation is large during the recovery period. The state of hypoventilation may sometimes persist during the early part of recovery in patients with an inadequate maximum breathing capacity. However, persistent hypoventilation during exercise and early recovery is also one of the most important evidences of inadequate response of the respiratory centers to the stimulus of a higher arterial $p\text{CO}_2$ (medullary centers) or a low arterial $p\text{O}_2$ (carotid body).

V. *Respiratory Gas Exchange at Rest, during and after the Standard Exercise*

A. *Carbon dioxide output.* The data in our control studies confirm the well known parallelism between CO_2 output and ventilation. They need no further emphasis.

B. *Oxygen consumption.* As seen in table 1 and 2 the mean minute oxygen consumption per square meter of body surface agrees with the standard basal metabolic figures in all but the youngest female group, in which it is lower. In confirmation of the work of Robinson, a significant negative correlation can be demonstrated in the male group between age and the resting oxygen consumption: $r = -0.478$ ($p = 0.004$). It is of interest that the figure for the mean oxygen consumption per square meter of body surface during the minute of standard exercise is practically identical for all 6 groups, indicating the same expenditure of energy per unit of body size. This finding adds to the validity of this type of exercise test. It will be seen also that the figures of mean oxygen consumption per square meter of body surface area during the 5 minutes of recovery are, for males and females, in close agreement. However, the range of variation of these figures is so large that the significance of differences between normal and pathological cases may prove to be of doubtful statistical value. Physiological consideration concerning variations in these figures of total oxygen consumption during the 5 minutes of recovery, which may be used to calculate the partial oxygen debt, in a manner somewhat similar to Nylin's (41) would therefore appear to be devoid of a sound statistical basis.

In normal subjects with an adequate total ventilation, and efficient alveolar ventilation, and a normal arterial oxygen saturation, the increase in oxygen consumption from rest to exercise is related to (a) an increase of pulmonary blood flow and (b) a reduction in the oxygen content of the venous blood returning to the right heart due to greater tissue oxygen demands. The maximum breathing capacity is so large that ventilation is never a limiting factor even during the most severe type of exercise. In subjects with pulmonary disease a correct interpretation of the changes in oxygen consumption involves more than considerations limited to tissue metabolism and circulatory response to exercise, especially when the picture is complicated by actual anoxia. Such additional factors include (a) limitation of the ventilatory volume due to low maximum breathing capacity, (b) inadequate distribution of inspired air, with the resulting irregular alveolar ventilation, and (c) an abnormally high diffusion gradient across the alveolar capillary membrane.

C. *Rate of oxygen removal.* The rates of oxygen removal during rest and the standard exercise are tabulated in table 10, together with figures calculated from observations made by other authors. On the whole, the values at rest check with those of Robinson remarkably well; although the decrease of the rate of oxygen removal in the middle age group is more striking in his data than in ours.

The values observed during our type of exercise should not be compared too closely with the values reported in other studies where a steady state was ob-

blood of normal subjects remains relatively constant at rest (table 3). Following the standard exercise test, the arterial blood of the normal subject usually shows a perceptible drop of carbon dioxide content but little change in the $p\text{CO}_2$ and only a slight decrease in pHs.

Sometimes variations may occur in normal subjects due to differences in physical training. In patients with pulmonary disease at least three adjustments may take place: (1) a low CO_2 tension and high pHs may develop as the result of hyperventilation due to excessive stimulation of the respiratory centers, (2) a high CO_2 tension and normal pHs may be associated at rest and during exercise with a significant degree of hyperventilation, (3) a high CO_2 tension and low pHs may be observed at rest and during exercise with hypoventilation. The finding of an increased carbon dioxide content and tension, in spite of hyperventilation, indicates serious impairment of the alveolar function. If the same finding obtains without hyperventilation, it suggests in addition a poor response of the respiratory centers.

VII. Return of Pulse Rate to the Resting Value during the Five Minute Recovery Period

The time required for the pulse rate to return to resting values following a standard exercise has been considered by many observers to be an important observation in the evaluation of the state of the circulation in subjects suffering from cardiac and circulatory disease (42, 43). In patients with pulmonary disease it might have been expected that a similar correlation would be found. In fact, in this series no significant correlation could be demonstrated between the time required for the pulse rate to return to resting values and either the total oxygen consumption, the changes in rate of oxygen removal, and the degree of arterial blood oxygen saturation during or following the 30 steps exercise test. Whether an increased pulse rate is of importance in detecting a circulatory factor in subjects with pulmonary disease still remains an open question.

VIII. Bronchspirometry

The importance of bronchspirometry consists in a comparison of the performance of each lung relative to the other. In normal individuals the vital capacity, the ventilation, and the oxygen intake of the right and left lung are respectively 55 per cent and 45 per cent of the total vital capacity, ventilation, and oxygen intake (44).

It is not unusual to observe during the test that a grossly abnormal lung contributes its normal percentage to the total ventilation. In these instances, the tidal air in the diseased lung encroaches much more on the vital capacity than in the opposite lung. Of far greater interest are the variations in the relative oxygen intake of each lung. These can be interpreted as an indication of the relative circulation of blood through each lung, providing that (a) the inspired air contains a concentration of oxygen sufficiently high to make up for alveolar distribution and alveolo-capillary diffusion abnormalities and (b) the arterial blood is fully saturated with oxygen.

IX. Infusion Test

Analysis of the data upon which the statistics in table 5 were calculated indicates that in normal subjects (a) the venous pressure usually does not rise towards the end of the infusion more than 30 mm. of saline with a final maximum height not over 115 mm. of saline, (b) the vital capacity decreases less than 10 per cent, and (c) the circulation time remains constant within 2 seconds.

A decrease in the vital capacity of 10 per cent or more indicates congestion of the pulmonary circuit. Rise of venous pressure is a measure of systemic venous congestion. However, a significant rise of venous pressure has also been observed in some patients with vasomotor instability, as for example in circulatory asthenia. A prolongation of the circulation time is a more reliable indication of circulatory dysfunction.

The test actually imposes only a slight additional load on the circulation and therefore will not demonstrate abnormalities in cases of mild or moderate diminution in cardiac reserve; for instance in 32 patients with compensated organic heart disease no significant difference could be demonstrated by comparison with a control group. This test will frequently bring out a state of incipient congestion in either the systemic or the pulmonary circuit, whether such congestion is primarily of circulatory or of pulmonary origin. Thus significant mean changes in the venous pressure, vital capacity, and circulation time measurements are encountered not only in groups of patients with decompensated organic heart disease but also in patients with chest deformities, fibrothorax, and various types of chronic pulmonary disease. Even in such groups, however, the wide scatter of values makes a negative test of doubtful significance.

For all these reasons one may conclude only that a positive test is of considerable clinical value in the evaluation and the therapeutic management of certain selected cases.

(b) residual air and index of intrapulmonary mixing, by collection of expired air during oxygen breathing; (c) measurement of respiratory gases, ventilation and arterial blood, at rest and following a standard exercise; in certain instances (d) other special measurements such as bronchspirometry, and the infusion test.

4. Values of various pulmonary functions in normal subjects are compiled from our data and from those of other investigators, and formulae for the prediction of normal values, according to sex, age, and body surface area, are presented.

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INFECTIOUS HEPATITIS¹

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INTRODUCTION

The identification of the terms catarrhal jaundice, infectious hepatitis, hepatitis epidemica, and acute yellow atrophy of the liver as descriptive appellations of a single disease entity was suggested many years ago by the clinical and epidemiologic observations of several investigators (1-8). Until recently, the classification and description of this entity were on a clinical-pathologic basis. The early concept of Virchow (9) that catarrhal jaundice resulted from the obstruction caused by a plug of mucus in the ampulla of Vater was altered by the descriptions of Eppinger (10) and later by Rich (11) of diffuse hepatocellular necrosis in patients with this disease at necropsy. Subsequent observations by Roholm (12), Iversen (12), Dible et al. (13) and others (14, 15) of specimens of the liver obtained by biopsy from patients with infectious hepatitis supported the idea that the essential lesion in this disease is mainly concerned with inflammatory and degenerative changes in the hepatic parenchymal cells. However, in spite of the unequivocal evidence assembled throughout the years, Mallory (15) has called attention to the fact that medical opinion in general continued to accept the early concept of Virchow (9), and only recently has the actual pathogenesis of the disease been generally appreciated.

During the years of World War II, the importance of infectious hepatitis as an epidemic disease initiated numerous studies directed towards clarifying certain problems hitherto unsolved. In addition to the amplification of knowledge of the natural history of the disease, a new concept of its viral etiology has been evolved. It has also been suggested that there may be variant forms of viral hepatitis which, although clinically and pathologically indistinguishable, have certain apparent differences. Whether these differences indicate actually different diseases or variant forms of a single disease is, as yet, undetermined.

Terminology. At least two forms of viral hepatitis are now recognized: (1) *infectious hepatitis* (catarrhal jaundice, hepatitis epidemica, infective hepatitis) and (2) *homologous serum hepatitis* (late post-arsphenamine jaundice, syringe jaundice, transfusion jaundice, yellow fever vaccine jaundice). The former term, *infectious hepatitis*, is reserved for the sporadic or epidemic forms of the naturally occurring disease. The latter term, *homologous serum hepatitis*, is used to designate that form of hepatitis produced in patients by the parenteral injection of human blood or its products containing a virus of hepatitis.

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It is obvious that the arbitrary definition of a disease on the basis of route of inoculation of the causative agent is artificial and likely to cause confusion. *Homologous serum hepatitis* is admittedly a poor term, particularly in view of recent experimental work which has described at least two apparently different strains of virus which cause hepatitis in man (16, 17). Since both of these strains of virus may be transmitted experimentally by parenteral inoculation, they conceivably could both be described as homologous serum hepatitis virus, although certain differences in behavior in man suggest that, while they may be closely related, they are not identical. To avoid this difficulty in nomenclature, Neefe (18) has recently suggested the terms, "Virus Hepatitis I. H." to designate the naturally occurring disease, and "Virus Hepatitis S. H." to refer to the artificially transmitted homologous serum hepatitis.

The first section of this paper will deal with infectious hepatitis, the naturally occurring disease, and the second section with homologous serum hepatitis and its possible relationship to the former disease.

INFECTIOUS HEPATITIS

History

Infectious hepatitis has a long and well-established record of importance in both civilian and military medical history. Epidemics of the disease were apparently recognized by ancient Greek physicians, and descriptions of outbreaks from various parts of the world have appeared with increasing frequency since the middle of the 18th century. Blumer (5) reported that 11 outbreaks had been described in the American literature from 1812-1886; 51 outbreaks from 1886-1920; and from 1920-1922 a greatly increased number were recorded, with more than 200 in New York State alone. It has been repeatedly observed that when troops are concentrated during war, this disease is likely to occur. It was present among Napoleon's troops in Egypt, and epidemics have occurred in this country during every war with the possible exception of the War for Independence. 71,691 cases appeared among Union troops during the Civil War (19). During World War I, it was prevalent among British (20-23) and French (24) troops in the Mediterranean area; and during World War II, it was a major cause of loss of time in both Allied (25-31) and Axis (32-37) forces. Hundreds of thousands of cases occurred in both civilian and military populations in many widely separated parts of the world (38-54); thousands of cases with jaundice are known to have occurred among American troops alone.

Early etiologic investigations in this disease were concerned with *Leptospira icterohemorrhagiae*, and for some time confusion existed concerning the possible rôle of this organism in the disease. During World War I, emphasis was placed on the possibility of an etiologic relationship between various strains of salmonellae and infectious hepatitis. The recovery of such organisms from the blood or feces of some patients with jaundice, as well as the development of antibodies in their blood to certain strains of salmonellae, suggested a possible etiologic association (24, 55-58). In the light of present knowledge, it is probable that

such observations indicated the simultaneous occurrence of two diseases whose manner of spread (the intestinal-oral route) was the same. Of interest in this regard is a recent report (59) of bacteremia with *S. cholerae suis* in 2 volunteers experimentally infected with infectious hepatitis. These men lived in institutions where infections with salmonellae were not uncommon, suggesting the possibility that hepatitis may predispose a patient to the acquisition of secondary infection with these bacteria.

The modern concept of the viral etiology of infectious hepatitis is a product of investigations carried on during World War II. Although the virus has not been "isolated" in the sense that it has been adapted to laboratory animals, it has been studied in volunteers. From such experiments, certain information has become available concerning properties of virus and possible ways of transmission and prevention of disease.

EXPERIMENTS IN TRANSMISSION OF VIRUS TO ANIMALS

In 1938, Andersen and Tulinius in Denmark (60) described the transmission of spontaneously appearing porcine hepatitis to young pigs on a deficient diet. In addition, they reported that duodenal fluid obtained from patients in the acute phase of epidemic hepatitis produced hepatitis in young pigs 2 to 5 days following ingestion. The hepatitis so produced was also said to be transmissible from pigs to rats by feeding infectious materials. An effort was made to establish an etiologic relationship between porcine and human hepatitis on the basis of experimental and epidemiologic data. Limited attempts to confirm this work have been unsuccessful up to the present (61, 62).

Since then, several attempts to transmit the virus² of hepatitis to embryonating hen's eggs (63, 64) and to a variety of animals such as hamsters (63), chimpanzees (65), guinea pigs, white mice, rabbits, kittens, gerbils, baboons, rhesus monkeys and other species such as *cercopithecus*, *erythrocebus* and *colobus* monkeys have been unsuccessful (66). Unconfirmed reports have described the propagation of this virus in embryonating eggs (67-69) and canaries (70, 71). More recently, MacCallum and Miles (72) have reported the production of hepatitis in rats on a deficient diet, by feeding duodenal fluid from patients in the acute phase of the disease. Subsequent experiments in their hands failed to confirm earlier observations. The spontaneous appearance of hepatitis in colonies of supposedly healthy mice (73, 74) and in puppies (75) has been described. There is no evidence, as yet, that any relationship exists between the agents causing these affections and hepatitis in man.

Up to the present, the identification of hepatitis virus is determined by its transmission to volunteers, and none of those strains of virus claimed to have been propagated in animals or embryonating eggs has been transferred to man, fulfilling this criterion.

² German (69) workers have described the virus of infectious hepatitis as being a polyhedral body with a diameter of the order of 180 millimicrons when visualized by means of the electron microscope. This report has not been confirmed.

EXPERIMENTS IN TRANSMISSION OF VIRUS TO VOLUNTEERS

Much of the information available about the virus of infectious hepatitis has been derived from experiments in its transmission to volunteers. The number of volunteers employed in any individual experiment is, of necessity, small, so that the results must be interpreted with caution. In general, the positive results are of greater value than the negative. However, during the past five years, the accumulated experience of a number of investigators employing volunteers has revealed certain observations which have been consistent. When all the work is tabulated, it is evident that certain similar results have been obtained often enough by different groups to lend an increased validity to individual findings (Table I).

Voegt (76) was the first to report the transmission of infectious hepatitis to volunteers by feeding duodenal fluid and blood obtained from patients in the acute phase of disease. In 1943, Cameron (77) described the transmission of hepatitis to 6 volunteers by the intramuscular injection of blood obtained from a patient early in disease. In 1944, MacCallum and Bradley (78) in England and members of The Neurotropic Virus Disease Commission of the Army Epidemiological Board in the United States (79) were successful in transmitting the disease to volunteers by feeding feces of patients in the acute phase of infectious hepatitis. Subsequently, it was demonstrated that the etiologic agent is filtrable through an L2 Chamberland or Seitz E K filter, resistant to heating to 56° C. for at least 30 minutes, and transmissible to man in serial passage by feeding or parenteral inoculation of infectious material (80). It withstands chlorination, viz., 1 part chlorine residual per million for 30 minutes (81, 82), and remains active in materials frozen 1 to 1½ years (18) but not for 3 years (282) at -10° to -20° C. Another strain of virus was inactive after storage at dry ice box temperature for 32 months (63, 282).

The infectivity of both urine and nasopharyngeal washings has been incompletely investigated. Contradictory results have followed the ingestion of urine by volunteers. Voegt (76), and Findlay and Willcox (83) reported positive results, while MacCallum and Bradley (78), Havens (84), and Neefe and Stokes (81) failed to transmit the disease in this way. More recently, Findlay (284) has suggested that the apparent infectivity of urine in his experiment was due to the presence of erythrocytes associated with urinary bilharziasis. The results of testing nasopharyngeal washings have been negative (78, 81, 84) with one possible exception (78). The number of volunteers employed in these latter experiments is small and, in addition, it is possible that both urine and nasopharyngeal washings were obtained from the patients at a time when insufficient virus to be infectious was present in such materials. It is evident that no conclusions concerning the infectivity of either urine or nasopharyngeal washings can be reached from the observations made up to the present.

Since most experiments were done with infectious material obtained from patients in the acute phase of disease, little is known about when virus appears in the blood or stools of such patients or how long it remains there. The latter is of particular importance in relation to the question of whether patients with

TABLE I

Results of Administration to Volunteers of Materials Obtained from Patients in the Acute Phase of Infectious Hepatitis

INOCULUM	AUTHORS	YEAR	ROUTE	NUMBER OF VOLUNTEERS		INCUBATION PERIOD
				Ino-cu-lated	Jaun-diced	
Feces	Voegt (duod. fl.) (76)	1912	O	4	1*	days 28
	MacCallum & Bradley (78)	1944	NP	26	3	27-31
	Havens et al. (70, 80, 84, 121, 124, 278)	1911-46	O	12	9	15-27
	Neeft et al. (123)	1944-46	O	46	25	18-37
	Neeft et al. (123)	1944-46	P	3	0	
	Findlay & Wilcox (83)	1945	O	18	7*	17-28
Serum	Voegt (blood) (76)	1942	O	?	1	?
	Voegt (blood) (76)	1912	P	?	1	19?
	Cameron (77)	1943	P	6	6	30 & 30+
	MacCallum & Bradley (78)	1944	P	6	3	64-92
	Havens (70, 80, 84, 121, 124, 278)	1944-46	P	17	8	20-31
	Havens (70, 80, 84, 121, 124, 278)	1944-46	O	8	7	21-34
	Oliphant (274)	1944	P	21	4	85-106
	Francis et al. (85)	1945	P	8	4	35-43
	Neeft et al. (123)	1945	P	6	1	35
	Neeft et al. (123)	1946	O	3	2	26 & 33
Naso-pharyngeal washings	MacCallum & Bradley (78)	1944	NP	16	0	
	Neeft et al. (123)	1945	NP	8	0	
	Havens (84)	1946	NP	3	0	
Urine	Voegt (76)	1942	O	?	1*	?
	MacCallum & Bradley (78)	1944	NP & O	19	0	
	Findlay & Wilcox (83)**	1945	O	6	3*	?
	Neeft & Stokes (81)	1945	O	7	0	
	Havens (84)	1946	O	3	0	

NP = nasopharyngeal; O = oral; P = parenteral.

Although the occurrence of non-icteric hepatitis is recognized, only jaundiced cases, in whom the diagnosis could be definite, are recorded here.

* No definite statement of jaundice.

** In a subsequent publication (284) Findlay has suggested that the apparent infectivity of urine in his experiment was due to the presence of erythrocytes associated with urinary bilharziasis.

relapse or chronic hepatitis are infectious, or whether persons who have made a complete recovery may remain carriers of virus. A limited number of experiments employing a few volunteers have been performed to investigate the period

of infectivity of patients with infectious hepatitis (Table II). Virus has been demonstrated in the blood 3 days before the appearance of symptoms (85), and as late as 8 days after the appearance of jaundice (79). A single attempt to detect virus in the blood of a patient half-way through the incubation period of experimentally induced infectious hepatitis was unsuccessful (84). Attempts to recover virus from the blood and feces 1 month (84) after onset, and from the feces 3 months (86) after disappearance of jaundice have also been unsuccessful. Neefe et al. (87) have tried to determine the infectivity of patients complaining of symptoms 6 to 9 months after the onset of hepatitis. Specimens of liver, obtained by biopsy, as well as blood and feces from such patients, were fed to volunteers who developed certain vague symptoms and slight alterations of tests

TABLE II

Results of Administration to Volunteers of Materials Obtained from Patients in Various Stages of the Incubation Period and Convalescence of Infectious Hepatitis

AUTHORS	YEAR	INOCULUM	DAY OF DISEASE MATERIAL OBTAINED	NUMBER OF VOLUNTEERS	
				Inoculated	Jaundiced
Havens (84).....	1946	F	-15	3	0
Francis et al. (85).....	1945	S	-3	8	4
Havens (84).....	1946	F & S	25+ to 31+	10	0
Neefe et al. (86).....	1945	F	21 post-jaundice	7	0
Neefe et al. (87).....	1947	Liver	180+	5	0
Neefe et al. (87).....	1947	S	106+ to 367+	5	0
Neefe et al. (87).....	1947	F	92+ to 342+	5	0

F = feces; S = serum.

(+) = after onset.

(-) = before onset.

of hepatic function. The results were not clearly defined, and it is still not definitely known whether such patients harbor virus or may be regarded as infectious.

EPIDEMIOLOGY

Unfortunately, infectious hepatitis has not been reportable to public health authorities in most places until very recently, so that much of the information available has come from descriptions of epidemics large enough to demand attention. Sweden and Denmark have been exceptions to this, and the disease has been notifiable in those countries since 1928. A great deal of information on statistical epidemiology is available from their records (Fig. 1).

Excellent descriptions of the clinical epidemiology of infectious hepatitis in both civilian (45, 88-96) and military (28, 30, 31, 97-103) populations are recorded. Although the age distribution is different, attention has been called (30) to certain similarities between the epidemiologic patterns of the disease in both groups. Much new information has been obtained during the years of

World War II, particularly in relation to the causative virus and possible ways of transmission and prevention of disease. The opportunity to observe the response of large numbers of susceptible young adults when placed in an environment where the disease is prevalent has been of value in defining more accurately the natural history of this disease. Up to the present, however, certain inherent difficulties in the investigation of infectious hepatitis, i.e., the lack of a susceptible laboratory animal as well as a specific serologic test, make impossible the solution of many of the problems associated with the disease.

Geographic Distribution. The accumulated records of outbreaks indicate that infectious hepatitis is widespread and probably occurs throughout the world (104). Reports from such widely separated areas as the United States (5, 81), Scandinavia (45, 90), North Africa (105), Palestine (52), India (106) and the

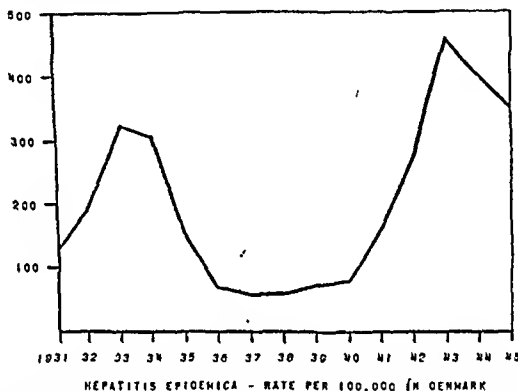


FIG. 1. (Havens, W. P., Jr.: Report on Epidemic Hepatitis in Denmark. August-September 1946. Conference on Liver Injury, Josiah Macy, Jr., Foundation. Transactions of the Fifth Meeting, 1946, p. 107.)
(Courtesy of the Josiah Macy, Jr., Foundation.)

South Pacific Islands (103) attest its generalized distribution. During the years 1942-1947, the disease reached epidemic proportions in many parts of the world, and was a major cause of sickness in both Allied and Axis forces (107). Certain areas have a well-established record of high frequency of this disease; in particular, the Mediterranean littoral has had a prolonged and high endemicity with severe epidemics among foreign troops stationed there during World Wars I (20-24) and II (25-28, 30, 31, 34, 37, 66, 102). At present, infectious hepatitis is not known to be limited geographically by any particular climatic conditions; however, it is believed that this disease is most likely to be prevalent where sanitary conditions are poor. The definition of the exact geographic distribution of the disease requires a specific means of identification of virus as well as a method of measuring immunity in order to determine the degree of past experience of various populations with infection.

Season. Although infectious hepatitis may occur at any time throughout the year, the prevalence of epidemics in the autumn and early winter months, with a decline in incidence during the spring and summer, has been observed in many different parts of the world including the United States (5), Scandinavia (45), England (89) and the Middle East (102). The exact explanation of this seasonal trend is not known. Kligler et al. (52) have suggested that it may result from the crowding which frequently occurs at this time of the year, and suggest the possibility that closer *personal contact* may account for the higher incidence. The fact that the disease apparently flourishes under poor sanitary conditions adds support to this concept. Under proper conditions, however, epidemics may occur at any time, and Kligler et al. (52) have described an outbreak among young immigrants to Palestine, reaching a peak in June.

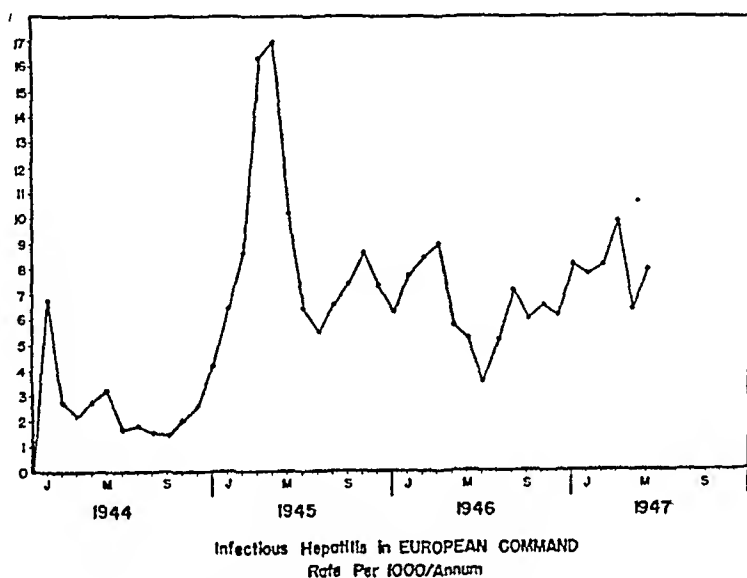


FIG. 2. INCIDENCE OF INFECTIOUS HEPATITIS AMONG AMERICAN TROOPS IN THE EUROPEAN COMMAND

In some places, hepatitis occurs throughout the year with little apparent seasonal variation in incidence. Such endemicity is said to be characteristic in Chile (Ducci (108)). Of interest in this regard has been the pattern of spread of the disease among American troops in Germany during the years 1944-1947 (Fig. 2). The rate of hepatitis rose sharply to epidemic proportions in 1945 during the early winter and declined in the spring. Since then, the seasonal variation has been slight, although somewhat higher rates have prevailed during the winter months of 1946 and 1947. In addition, localized outbreaks have been uncommon, and the disease has apparently been diffusely spread throughout the command. The exact explanation of this endemic pattern is unknown. The possibility exists that the artificial transmission of virus by improperly sterilized stylets, needles or syringes employed in such procedures as obtaining blood for laboratory tests or administering penicillin or vaccines might be operative in producing such a regular incidence of disease (109).

Age. Infectious hepatitis is primarily a disease of childhood in the civilian population, although it may occur at any age. Epidemics involving adults primarily have been reported, but the experience of most civilian outbreaks records 65 to 100 per cent of the cases in children (110). A comparison of the attack rates for this disease in 3800 inhabitants of an East Suffolk town with the attack rates in the children of the same community revealed the highest rate to be 6.0/1000 for the total population compared to a rate of 54.5/1000 for school children (91). Children under 15 are most susceptible, and the highest attack rates have been seen in the age group 10 to 14 (Sclander (90)). The disease is thought to be uncommon in children under 5 years, although it is possible that the mildness of infection in this age group may cause it to escape detection.

Under proper conditions, young adults up to 30 are very susceptible, as indicated by the high incidence of disease among troops in certain areas during World War II (30, 31). After 30, resistance to the disease apparently increases (31, 52). Among American troops in the Mediterranean area, there was no marked difference of age incidence under 30 years. From 30 to 33 years, however, the incidence was one-half that in troops under 30, and men over 33 had only one-third the incidence of infectious hepatitis of men under 30 years (31). It is not determined whether immunological or constitutional factors condition this response. In addition, the question has been raised as to whether difference in exposure among younger and older troops, as determined by the fact that men under 30 were more likely to serve in front line battle, might not influence this apparently sharp difference in susceptibility. That such factors are not always operative in determining this difference is suggested by the observation of Gauld (31) that headquarters personnel at times had a higher incidence of disease than combat troops.

Epidemics. In civilian life, both explosive and slowly developing epidemics may occur, although the usual experience during peace time is the occurrence of straggling outbreaks which may involve several hundreds (usually children) during the course of 2 to 4 months. Family and institutional outbreaks are common. In the former, it is usual for hepatitis to occur in one member of the family, followed in 2 to 4 weeks by subsequent cases (93). Institutional epidemics, particularly in schools and asylums, may involve as many as 80 per cent of certain groups.

Of interest is the epidemic pattern in military populations in which the predominant age group consists of young adults. Numerous examples of 20 to 40 day incubation periods of contact cases in military units are similar to the classical family outbreaks during civilian epidemics (McFarlan (30)). It has been observed especially that when groups of such young susceptibles enter an area where infectious hepatitis is endemic, as in the Mediterranean littoral, large outbreaks frequently make their appearance within the next 1 to 2 months. Involvement of 40 to 50 per cent of a command is not unusual in such military outbreaks. Two factors which may possibly increase such epidemicity have been suggested by Kligler et al. (52): (1) the continued introduction of susceptibles and (2) the wide dissemination of virus by movement of troops.

Explosive outbreaks may occur, but the pattern usually observed is a slow spread of disease over a period of 3 to 4 months. McFarlan (30) has suggested that the straggling course is characteristic of a mildly infectious disease which is spread by contact and has a relatively long and varied incubation period. Gauld (31) has described several such outbreaks in which cases appearing at the beginning and end of the epidemic are 1 to 3 weeks apart, while at the height of the outbreak several cases appear simultaneously. No exact reason for the frequently observed wide scatter of cases is known, although the possibility of sub-clinical cases or carriers of virus being operative in the dissemination of the disease must be considered (31) (Fig. 3).

The spread of hepatitis among troops has presented some interesting problems. A comparison of attack rates for this disease in England and the Middle East showed that in British troops in the Middle East in 1942-1943 rates ranged from 9 to 35.2/1000, while in similar troops in England in 1943-1944 rates ranged from

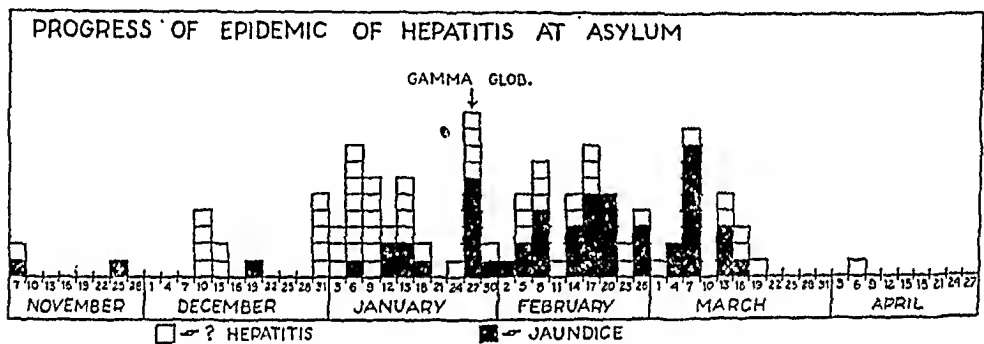


FIG. 3. AN OUTBREAK OF INFECTIOUS HEPATITIS IN WHICH GAMMA GLOBULIN WAS GIVEN PROPHYLACTICALLY. THE PROGRESSION OF THE EPIDEMIC WAS ALMOST COMPLETELY CONFINED TO THE UNPROTECTED GROUP

(Havens, W. P., Jr., and Paul, J. R.: Prevention of Infectious Hepatitis with Gamma Globulin. *J. A. M. A.*, 129: 270, 1945.)
(Courtesy of the Journal.)

0.2 to 0.5/1000 (30). Variations in incidence of disease in various groups have also been cited; British officers in the 1942 epidemics in the Middle East had attack rates 4.7 times as great as enlisted men (McFarlan (30)). Gauld (31) has reported a similar situation among certain groups of American troops. Over 30 years of age, the incidence of hepatitis was 3 times as great in American officers as enlisted men; while under 30 years, the incidence was only 40 per cent higher in officers. The explanation of the age differential is not clear.

The bulk of evidence at present suggests that variations in pathogenicity and infectivity of virus, in addition to certain environmental factors such as crowding and poor sanitation in areas where the disease is highly endemic, may be important conditions in determining variations of incidence of disease (McFarlan (30)). Attempts have been made to explain the higher incidence in officers as a group on the basis of such environmental factors. The enlisted men used individual mess kits and were responsible for their cleanliness; the officers messed together and had far more possibility of contamination of their food by carriers

or sub-clinical cases working as food-handlers. Environmental factors have also been evoked to explain the significantly higher incidence of hepatitis in certain epidemics among headquarters personnel as compared to combat troops (31). The former are usually more crowded together, and person-to-person contact is more common. The importance of this is suggested particularly if there is a good deal of virus in the environment.

Transmission of Disease. The exact way or ways in which infectious hepatitis spreads are not known, although there is epidemiologic and experimental evidence to indicate that some form of person-to-person contact is frequently operative. It is not unlikely that more than one manner of spread are effective, and that epidemics result from different combinations of various factors.

The fact that virus is in the feces and may be transmitted experimentally by feeding such infectious materials suggests that the intestinal-oral route may be of considerable importance. The increased incidence in periods of the year (autumn and winter) when crowding is common, together with the oft-repeated history of association of this disease with poor sanitation, lends support to this concept. It is of particular interest in this regard that when epidemics of this disease occur in institutions for mentally deficient children, the highest rates occur in groups having the lowest intellectual level, among which it is most difficult to maintain high standards of sanitation (110).

As long ago as 1916, Kartulis (111) suggested that infectious hepatitis might be spread by contaminated water. A number of presumably water-borne (31, 81, 112, 113) outbreaks, as well as food-borne (114) and milk-borne (115) epidemics, have been described, although there is no evidence that these are the most common modes of transmission. Of particular importance was the demonstration by Neefe and Stokes (81) of hepatitis virus in water obtained from a well in a children's camp in Pennsylvania during an epidemic of the disease. Volunteers contracted infectious hepatitis following ingestion of water from the well which was proved to have fecal contamination. Virus was also obtained from the feces of patients with infectious hepatitis in the camp, producing the disease in volunteers who ingested such infectious materials.

Attention has also been directed to the respiratory route as a possible way of spread of infectious hepatitis. Emphasis has been placed on this possibility because of the clinical observation of symptoms and signs of disease of the upper respiratory tract in a certain percentage of patients at the onset of infectious hepatitis. This varies with epidemics, and the exact significance cannot be defined. The increased incidence of infectious hepatitis during the fall and winter months when respiratory disease is more prevalent has been cited in support of this concept (31). In addition, circumscribed outbreaks in small groups occupying the same sleeping quarters under crowded conditions have been described as supportive evidence for the spread of disease in this manner. While the occurrence of outbreaks under such living conditions suggests that some form of person-to-person contact may frequently be responsible for the spread of hepatitis, there is no evidence which incriminates the respiratory rather than the gastrointestinal route. Experimentally, there is no conclusive

evidence to support the concept of the respiratory route of transmission, but it is important to note that only a limited number of experiments have been performed on a few volunteers, so that such negative results are not definitive.

The possibility of transmission by insects, either by biting or by mechanical transfer of infectious materials, has received attention. The former method requires consideration in view of the fact that this disease may be transmitted to man by the parenteral inoculation of as little as 0.01 cc. of infectious serum (84), which is well within the range of inoculation by certain insects. However, numerous epidemics occur in areas and seasons when no possible vectors are present. The latter possibility of mechanical transfer of infectious material has been advanced by Kirk (99) and Trussell (103) in their descriptions of outbreaks among New Zealand and American troops at El Alamein and in the South Pacific area.

Lastly, the possibility of artificial transmission of infectious hepatitis merits consideration. This will be discussed in greater detail in the section on homologous serum hepatitis. However, it is to be noted at this point that the presence of hepatitis virus in the blood of patients, its high degree of resistance to ordinary procedures of cleansing, and its infectivity by parenteral inoculation suggest the possibility that it may be transmitted accidentally more often than is recognized.

Immunity. The lack of a specific serologic test or of a susceptible laboratory animal makes it difficult to evaluate the immune response elicited in man following infectious hepatitis. However, epidemiologic evidence is supported by a limited amount of experimental data which suggest that a degree of immunity does follow infection. In civilian life, Pickles (89, 116) has called attention to the fact that long intervals occur between epidemics in villages. Lisney (91) also noted that villages in Leicestershire that had had epidemics before 1942-1943 were not involved in the widespread epidemic of that year.

The natural history of the disease is in accord with the concept that an attack confers immunity. Infectious hepatitis is primarily a disease of childhood and, although young adults are highly susceptible under proper conditions, the incidence of disease declines rapidly after 30 years of age. It is a common experience for epidemics to occur among susceptible immigrant populations when introduced into an area where the disease is endemic (52). In such situations, the low degree of susceptibility of the surrounding native population has been recorded (30, 52).

In questioning groups of young adults about past history of jaundice, the usual incidence is 3 to 5 per cent. However, this does not account for the patients with hepatitis without jaundice or the sub-clinical cases. The mildness of the disease in childhood (110) suggests the possibility that infection is far more common than usually suspected, resulting in subsequent relative immunity. In support of this is the original work of Stokes and Neefe (117), subsequently corroborated by others (118, 119), of the prophylactic value of normal human gamma globulin in this disease. When given during the incubation period, up to within 6 days before the onset of disease, passive protection is conferred.

The epidemiologic experience with infectious hepatitis in troops has added

further supportive evidence to the idea that immunity follows an attack (McFarlan (30)). Gauld (120) reported the incidence of infectious hepatitis as 42 per 1000 among seasoned American troops in the Mediterranean theatre (1944-1945), compared to an incidence of 109 per 1000 among reinforcements. "Reinforcements" were defined as troops who had entered the theatre after the winter epidemic of 1943-1944; "seasoned" troops were men who had been present in the theatre during this time. It has also been suggested that the rise in incidence of disease in one group paralleling a declining incidence in an associated group also indicates the possible development of resistance (30).

It is difficult to evaluate the clinical and epidemiologic data which indicate that second attacks of hepatitis may occur in 3 to 5 per cent of patients. Considerable variations may be observed in the second attack rates. Among civilian populations (Pickles (89), Lisney (91)), they are apparently uncommon,

TABLE III

*Results of Attempts to Demonstrate Immunity and Cross Immunity in Volunteers
Convalescent from Experimentally Induced Infectious Hepatitis*

AUTHORS	YEAR	CHALLENGE VIRUS	NUMBER OF CONVALESCENTS			NUMBER OF CONTROLS		
			Inocu- lated	Jaun- diced	Incubation Period	Inocu- lated	Jaun- diced	Incubation Period
					days			days
Havens (121).....	1946	IH*	0	0		12	8	21-30
Neeffe et al. (123).....	1946	IH	17	0		14	6	25-37
Neeffe et al. (123)	1946	HSJ**	4	2	101, 102	9	8	60-110

* Infectious hepatitis.

** Homologous serum jaundice.

ranging from none to 4 cases in two series of 1000 cases. Differences have also been pointed out by McFarlan (30) in studying the attack rates among comparable groups of troops. Of 77 men who contracted infectious hepatitis in the Middle East, 8 had had previous attacks. Of 82 men who contracted this disease in England, only 1 had been jaundiced before, and his first attack was 8 months before the second, suggesting the possibility of relapse. It is possible that immunity may not be solid, and that size of dose of infectious agent, as well as certain non-specific or environmental factors affecting the immunity of the host, may be operative. Of particular importance is the fact that it is not yet possible to determine whether such second attacks represent actual reinfection with the same virus or infection with another strain of hepatitis virus. It is also known that evidence of hepatic involvement with or without jaundice may occur in infectious mononucleosis so that it may be difficult to distinguish between this disease and second attacks of hepatitis.

Experimentally, there is a limited amount of data to indicate that immunity develops during recovery from hepatitis. Both Havens (121) and Neeffe et al. (122) showed that volunteers convalescent 6 to 9 months from experimentally induced infectious hepatitis were immune when reinoculated with the homologous

strain. In addition, Neefe et al. (123) showed that volunteers recovered from hepatitis experimentally induced by a strain of virus obtained from the stools of children with the disease in Pennsylvania were immune when inoculated with a strain of hepatitis virus obtained from the stool of a soldier who contracted the disease in Sicily (79) (Table III).

Evidence is now available that more than one strain of hepatitis virus may cause hepatitis in man. At least two strains of virus have been described as being immunologically distinct (16, 17, 123). These strains of virus have been termed *infectious hepatitis* and *homologous serum hepatitis*, and the differences between them will be discussed more fully in the section on Homologous Serum Hepatitis. However, it may be mentioned here that in a limited number of experiments, volunteers convalescent from hepatitis produced by one strain of virus were not immune when reinoculated with the other strain of virus (123, 124).

PATHOGENESIS

The most striking and apparent effect of hepatitis virus in man is on the parenchymal cells of the liver which may be involved in all degrees of inflammatory and degenerative changes. Unfortunately, too little is known about this virus to allow conclusive definition of the pathogenesis of the disease it causes. However, there is reason to believe that infectious hepatitis is a generalized systemic infection in which the alimentary tract is involved early. The presence of virus in the blood, with lymphadenopathy and splenomegaly in addition to chilliness, fever, malaise and headache early in the course of disease, supports this concept. Anorexia, nausea, vomiting and abdominal distress suggest that involvement of the gastro-intestinal tract may be an important feature of this disease. In support of this are the roentgenographic (125, 126) and gastroscopic (126, 127) findings, which have been described as evidence of gastro-duodenitis, in the acute phase of disease. Other objective evidence of involvement of the gastro-intestinal tract has been furnished by the demonstration at necropsy of regional lymphadenopathy with edema and phlegmonous changes in the stomach and small and large intestine in fatal cases (98, 128, 129).

PATHOLOGY

Excellent reviews of the development of knowledge of the pathology of infectious hepatitis have been made by Lucké and Mallory (128, 129). Attention has been called to the belated acceptance of the actual pathogenesis of this disease by general medical opinion in spite of the unequivocal evidence assembled in clinical, epidemiologic and pathologic observations (15). It was not until the technique of biopsy of the liver was developed by Iversen and Roholm (12, 130) that a method of examination of the liver of such patients in various stages of disease was possible. Application of this procedure by others, notably Dible, McMichael and Sherlock (13), Axenfeld and Brass (14), and Kalk (131), has amplified the understanding of the pattern of histologic response of the liver in various stages of disease. Mallory (15) has described the pathologic alterations

of the hepatic parenchyma as observed in 160 biopsies performed on 137 patients in various stages of non-fatal infectious hepatitis. During the acute icteric phase, the characteristic changes consisted of peri-portal infiltration with a predominance of mononuclear cells; intralobular infiltration with swelling of the reticulo-endothelial cells; lobular disarray; focal necrosis with acidophilic degeneration of hepatic cells; numerous mitotic figures and multinucleate cells; and dilatation of the biliary canaliculi with biliary thrombi. The lobular reticular framework is usually maintained intact (Dible et al. (13)), although the pattern may at times be distorted with areas of condensation apparent (Mallory (15)). Dible et al. (13) have described central lobular necrosis in certain patients with severe but non-fatal hepatitis. In specimens of the liver obtained from patients in the pre-icteric phase or from patients with sub-icteric or non-icteric hepatitis, qualitatively similar observations were made with the exception that biliary stasis was rarely evident.

The length of time before recovery is complete varies widely; complete regeneration of the liver may occur at the end of 1 to 2 months, although activity of disease may be present for several months. Mallory (15) described specimens of the liver obtained by biopsy from 20 normal convalescents, at periods ranging from 34 to 131 days after onset of disease. Seven were normal; 5 had minimal changes which were defined as of doubtful significance; 8 men, however, still had evidence of peri-portal and intralobular inflammatory infiltration with occasional areas of focal hyaline necrosis and fat vacuoles in the hepatic cells. The range of variation in the rate of recovery is demonstrated by the fact that in 2 patients examined on the 32nd and 33rd days, evidence of hepatitis had almost cleared, while another showed moderately severe activity on the 83rd day from onset.

Mallory (15) examined 3 patients with second attacks from 10 to 13 months after the first attack. No evidence of permanent damage from the first attack was found. Lucké (132) reported that 14 patients, dying from other causes 1 week to 14 months after acute hepatitis, revealed essentially normal hepatic parenchyma. Slight residual peri-portal infiltration (12, 13, 87, 131, 133) may persist for several months, but the exact significance of this is not determined. Dible et al. (13) suggest that it ultimately disappears in most cases, and it would appear probable that some persons making an apparently *normal* recovery from acute hepatitis have some abnormalities of hepatic structure without active hepatic disease.

In another group of patients in whom convalescence was interrupted by relapse or prolonged beyond the usually accepted period of recovery, Mallory (15) observed histologic changes in the liver similar to those found in the *normal* convalescent group. Of 16 patients with relapse, 3 of them were examined during the height of recrudescence, and the others at intervals of 24 to 130 days after the appearance of jaundice. Ten showed positive evidence of hepatitis, and 6 were doubtful. In 40 other patients classified as "delayed recovery," biopsies of the liver were obtained from 100 to 500 days after the onset of disease. In 15 patients, the specimens of liver observed were normal; 10 patients had doubtful changes; and 15 had changes characteristic of hepatitis, with peri-portal and

intralobular inflammation and focal hyaline necrosis. The duration and progression of such lesions are unknown, although the concept that a certain number develop chronic progressive hepatic disease is borne out by the experience of others.

The occurrence of multiple nodular hyperplasia, post-necrotic cirrhosis, and diffuse portal cirrhosis of the liver in patients who had contracted infectious hepatitis during outbreaks of the disease has been recorded (13, 131, 133-145). How often the transition from viral hepatitis to cirrhosis of the liver occurs is undetermined. Recent reports from Denmark (139, 142) have described a highly fatal form of chronic hepatitis occurring particularly in women beyond the menopause, and terminating in subacute hepatic necrosis in 6 to 18 months after onset. The relationship of this disease to epidemic hepatitis, which has been so prevalent in Scandinavia during the past few years, is undetermined, although there is no reason to believe that the chronic form of disease is not merely a more severe manifestation of hepatitis in an older age group. Bergstrand reported a similar situation in Sweden in 1930 (146). The relationship of sex and climacteric is unexplained. Krarup and Roholm (137) in 1941, by means of serial biopsy of the liver, described the development of chronic hepatitis with diffuse cirrhosis in 12 out of 49 patients. They question whether such a high percentage of cirrhosis might not be due to selection of cases, since only sicker patients reached the hospital. In addition, it is of interest that all but 3 of their patients were over 40 years of age, suggesting that the increased severity of disease which is known to characterize hepatitis among older patients might be a factor in producing the observed changes.

Subsequent similar studies by others have revealed a much lower incidence of such a progression of disease, so that the preponderance of evidence suggests that it happens infrequently. Thus, Dible et al. (13) described the development of cirrhosis in only 2 of 54 patients with non-fatal viral hepatitis. A third patient was suspected of having multiple nodular hyperplasia and cirrhosis clinically. Sherlock and Walshe (133) found no evidence of cirrhosis in 35 non-fatal cases, and Mallory (15) observed none in 89 non-fatal cases. Kalk (131) recently described the findings in specimens of the liver obtained by biopsy in 400 patients at varying intervals after acute hepatitis. Nine had multiple nodular hyperplasia; 2 had diffuse cirrhosis; 1 had post-necrotic cirrhosis; and 8 had splenomegaly associated with essentially normal-appearing hepatic parenchyma. Eight other patients had residual peri-portal fibrous infiltration which disappeared almost completely during the ensuing few months.

Lucké and Mallory (129) have described the pathologic changes in fatal cases of the disease and have divided them into two types: (1) the fulminant and (2) the subacute. In the fulminant form of disease in which death occurs within 10 days after onset, the liver is reduced in size, smooth and soft. The cut surface may have a "nutmeg" appearance. Massive central necrosis destroys the hepatic parenchyma uniformly and completely. Evidence of regenerative hyperplasia is lacking. The necrosis is autolytic, and the affected cells disappear quickly. An intense inflammatory cellular response of the mononuclear type is

present at the periphery of the lobule, particularly in the portal stroma. The lobular remnants contain numerous proliferated macrophages and erythrocytes, so that the liver may resemble a spongy framework distended with inflammatory cells and blood. Similar observations have been recorded by Wood and Black (147, 148).

In the subacute form, death may occur from 3 to 8 weeks after onset. The liver is often grossly deformed and smaller than normal. Red depressed areas are surrounded by yellow-green nodules. The cut surface may be granular. Destruction of the hepatic parenchyma is neither complete nor uniform, and varying degrees of regeneration are seen with the formation of nodules of hepatic cells without normal lobular architecture. The inflammatory response is less pronounced but is mononuclear in type and involves the stroma of the portal areas and the interlobular boundaries. The reticulum is apparently not involved except insofar as shrinkage is evident in areas where parenchyma cells have disappeared. Endophlebitis of the central and efferent veins is more evident in this form than in the fulminant form of disease. As regeneration occurs, the new cells are often large and multinucleate in type.

Extra-hepatic lesions include lymphoid hyperplasia; the spleen is frequently enlarged and congested with follicular hyperplasia. Ascites is common. Phlegmonous inflammation and hemorrhage of the stomach and intestinal walls are found in the subacute cases. The kidneys are often swollen, with evidence of fat storage in the fulminant cases and bile nephrosis in the subacute cases. The brain may reveal swelling of the ganglion cells with distortion of nuclei and, in cases of longer duration, there may be lymphocytic infiltration of the meninges and perivascular lymphocytic cuffing in the basal ganglions.

CLINICAL COURSE OF DISEASE

Numerous observations suggest that infectious hepatitis is primarily a disease of children during peace time (110), in contrast to the high prevalence among young adults during war (30, 31). Although complete proof of the identity of the diseases which appear both sporadically and in epidemics in children and young adults is not established, there is no reason to believe that they differ (110). Supportive evidence for this concept is furnished by the fact that in most epidemics among children, occasional cases appear in adults associated with them. Experimentally, additional support for this idea is given by immunologic data which reveal that adult volunteers, convalescent from hepatitis induced by a strain of virus obtained from the stools of children with the disease in Pennsylvania, were immune when inoculated with a strain of virus derived from the stool of a soldier in the acute phase of infectious hepatitis, contracted in Sicily (123).

Incubation Period. The incubation period is believed to range from 20 to 40 days, although there is evidence to suggest that it may be as short as 10 days in some outbreaks. When infectious hepatitis is produced experimentally in volunteers by feeding infectious materials, the average incubation period is about 25 days, although it may be as short as 15 days and, on a single occasion, was recorded as long as 85 days (79).

Juvenile Form. Characteristically, the disease is qualitatively similar although milder in children. The typical juvenile case begins abruptly with headache, fever and gastro-intestinal symptoms, of which abdominal pain and vomiting are the most common. Fever usually ranges from 99.6° F. to 102° F., declining after 4 to 5 days to normal, when jaundice appears. As soon as jaundice is evident, there frequently occurs a striking amelioration of symptoms; the appetite improves, and a sense of well-being returns. Jaundice lasts usually 10 to 12 days. Complications are uncommon, and death is rare.

Non-icteric Hepatitis. It is undetermined how often hepatitis without jaundice occurs. That it happens more frequently than is usually suspected is suggested by the occurrence of characteristic symptoms and signs of hepatic dysfunction

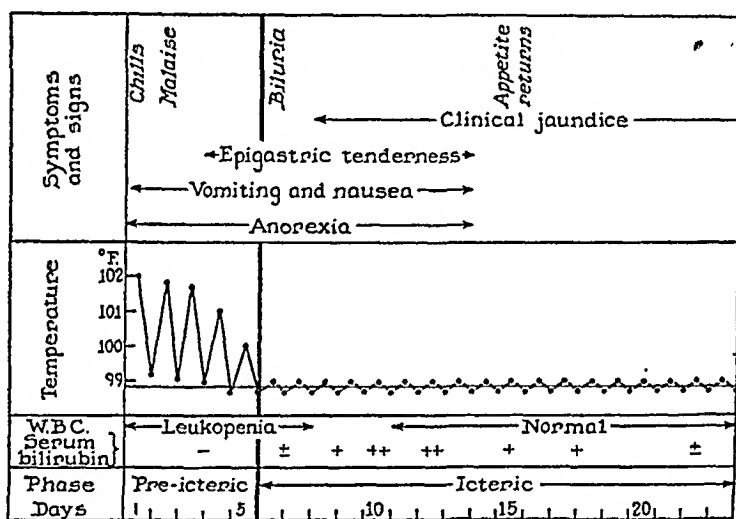


FIG. 4. SCHEMATIC DIAGRAM ILLUSTRATING THE CLINICAL COURSE OF AN AVERAGE CASE OF INFECTIOUS HEPATITIS IN AN ADULT

(Paul, J. R., and Havens, W. P., Jr.: Recent Advances in the Study of Infectious Hepatitis and Serum Jaundice. Trans. Assn. Am. Physicians, 59: 133, 1946.)
(Courtesy of Trans. Assn. Am. Physicians.)

without jaundice in a fair number of patients during epidemics of hepatitis when the diagnosis may be suggested by the epidemiologic situation and corroborated by tests of hepatic function (149). The liver may be enlarged and tender. Ordinarily, such disease is mild, although relapse with jaundice may occur. The importance of such patients as a source of infection in the community at risk is thought to be great.

Adult Form. Characteristically, the disease may be divided into two phases—pre-icteric and icteric—in a large percentage of patients. In two large series of military patients, 83 per cent had a definite pre-icteric phase while 17 per cent had jaundice as the first sign of disease (149, 150) (Fig. 4).

Anorexia is the most common and usually the first symptom in the pre-icteric phase. It is often insidious in appearance and particularly under conditions of military life, when rations are monotonous, such early aversion to food constitutes a valuable aid in diagnosis. As the pre-icteric phase progresses, malaise, weak-

ness and nausea become evident. Vomiting is less common early in disease, although later, just before jaundice appears, it frequently occurs. Abdominal discomfort, particularly in the epigastrium and right upper quadrant, is common and may be intensified by activity. Among troops, it was observed repeatedly that jolting the body in a truck or jeep caused abdominal pain. Disorders of bowel function vary in different outbreaks, although they are usually not a major complaint. Constipation is not uncommon, although in certain outbreaks diarrhea with flatulence occurs.

Of those patients with a definite pre-icteric phase, a variable percentage have fever. Since the determination of fever depends on objective observation, it is not easy to interpret the differences in incidence in various outbreaks. Under conditions of careful supervision, 100 per cent of a small group of volunteers, experimentally infected, had fever (110). In two large military groups (149, 150) fever was observed in 53 to 80 per cent of patients. In such patients, the onset of disease is usually abrupt with constitutional symptoms of malaise, headache, chill or chilliness, and generalized aches and pains. The temperature is often remittent with a daily elevation to 102° F., declining to normal over a period of 5 to 7 days. A certain number of patients have daily elevations of temperature to 104° F. for the first 2 or 3 days. Prostration is not uncommon in such patients, and photophobia and aching eyes, with pain on motion of the eyes, are associated complaints. As in the afebrile patients, anorexia, nausea, vomiting and abdominal distress appear early in the course of disease. A variable percentage of patients have symptoms of involvement of the upper respiratory tract.

The icteric phase usually begins 1 or 2 days after the temperature becomes normal, although jaundice may appear during the febrile period. The duration and severity of jaundice vary from a slight, fleeting icterus of the sclerae to prolonged deep jaundice which may persist as long as 4 months. The average duration ranges between 20 and 30 days. Fever is usually absent in this phase, although anorexia, nausea, vomiting and abdominal discomfort persist in all degrees of severity, with an average duration of 9 to 10 days. The symptoms may last as long as 3 to 4 weeks in the severely sick patient, while they may disappear in the mild case almost as jaundice appears. Less common but troublesome symptoms include somnolence, irritability and mental depression. Itching occurs in 12 to 21 per cent of patients and is usually associated with the period of increasing icterus, subsiding as jaundice diminishes (150, 151).

Physical examination during the pre-icteric phase frequently offers little assistance in diagnosis. Moderate injection of the conjunctivae, with tenderness to pressure on the eyeballs, may be present. In patients with symptoms of upper respiratory tract disease, the mucous membranes of the nose and throat are often suffused. Barker et al. (149) have called attention to the frequent occurrence of lymphadenopathy particularly in the posterior cervical areas. Palpation of the epigastrium often causes a sensation of nausea, and there may be tenderness in the right upper quadrant, particularly late in the pre-icteric phase when the liver may become palpable.

When jaundice becomes evident, the liver is palpable and usually tender in a

large percentage of patients. Ordinarily, tenderness subsides after 10 days, and the liver is no longer palpable after 2 weeks; however, both tenderness and hepatic enlargement may persist for several weeks in the severely sick patient. Splenomegaly, with tenderness to palpation, occurs in a small percentage of patients. Bradycardia is, at times, present in as many as 25 per cent of patients. Clinical evidence of pylorospasm, with dilatation of the stomach and abdominal distension, occasionally occurs. Relief of discomfort often follows vomiting of copious quantities of incompletely digested food.

Jaundice reaches its peak, on the average, in 10 days. During the period of increasing jaundice, particularly when it is severe, acholic stools are not uncommon. Loss of 5 to 10 pounds of body weight is a frequent occurrence, and more severely sick patients may lose 20 to 30 pounds. When jaundice reaches its peak, a striking amelioration of symptoms often occurs, with a return of sense of well-being. Appetite improves, and the average young adult, if urged, can eat as much as 4000 calories per day. Jaundice diminishes slowly, depending on the severity of the illness, age of the patient, physical status of the patient before hepatitis, and therapy received. In one group of military cases (150), the duration of jaundice was 6 days longer in men over 30 years than in men under 30 years. The period of hospitalization may range from a few days to several months, with an average of 50 to 60 days in military groups. Three months is a frequently accepted period for recovery, and it is not unusual for excessive activity during convalescence to produce an exacerbation of symptoms with abdominal distress. Barker et al. (149) have called attention to the danger of allowing men to return to duty too soon, and have advocated the use of a graded exercise tolerance test to determine when a patient is fit for full, strenuous activity.

The facts suggest that acute hepatitis usually is a mild, benign and self-limited disease. Although the morbidity may be high and the course of disease in adults often prolonged and debilitating, the over-all mortality rate is low—under 4 per 1000. When death does occur, the liver has the appearance usually described as acute or subacute hepatic necrosis.

Complications are uncommon; an occasional patient develops pneumonia; neurologic disorders such as lymphocytic meningitis and myelitis have been reported (152–155). A small percentage of patients develop seborrheic dermatitis and labial herpes (150).

In contrast to the majority of patients who apparently make an uneventful recovery, attention has been called to a small group of patients who have had either an interruption in their convalescence or a prolongation of symptoms and signs of disease beyond the usual period of expected recovery. Certain criteria for the diagnosis of relapse and chronic hepatitis make it possible to separate these patients into groups.

Relapse. Relapse is not uncommon, and rates ranging from $1\frac{1}{2}$ to 18 per cent have been reported (150, 156). The relapse may be a duplication of the original attack or may be more severe. In one carefully supervised group of patients (Kunkel et al. (157)), 14 per cent of 350 patients had relapse. Twenty per cent

of this group had no clinical signs or symptoms, and the evidence of relapse in these patients was furnished by serial determinations of hepatic function. Characteristically, relapse occurs after an apparently normal convalescence when the patient has been allowed to return to activity. Fatigue, anorexia, upper abdominal discomfort, with enlargement and tenderness of the liver, occur. The bromsulphalein dye retention test becomes abnormal, and the thymol turbidity test becomes more strongly positive. Clinical jaundice may or may not appear. Tenderness of the liver is said to be one of the most important physical abnormalities evident, while the degree of bromsulphalein dye retention is one of the most useful laboratory measures. Most patients with relapse make complete recovery, and Kunkel et al. (157) reported that only 2 of 49 such patients had evidence of chronic hepatitis at the end of 1 year after the onset of disease.

Chronic Hepatitis. It is apparent that a small percentage of patients with acute hepatitis continue to have evidence of hepatic disease long after the expected time of recovery. Characteristically, such patients have a classical history of acute hepatitis, followed by partial recovery, and then develop anorexia, lassitude, weakness and epigastric discomfort. The liver is often enlarged and tender, and the spleen is occasionally palpable. Jaundice is frequently not present but the bromsulphalein dye retention test, as well as the cephalin-cholesterol flocculation and thymol turbidity tests, is abnormal.

Attention has also been called to the fact that a certain number of patients have complaints of anorexia, fatigue and abdominal discomfort 2 to 24 months after the onset of hepatitis, at a time when only an occasional test of hepatic function is abnormal (158). The term "post-hepatitis syndrome" (159, 160) has been used to describe this situation, and an analogy to "effort syndrome" has been made by Sherlock and Walshe (133). The difficulty of correlating subjective and objective evidence of hepatic disease during convalescence is emphasized by these workers who performed biopsy of the liver of 20 such patients. The only changes in the liver were slight, residual, fibrous, peri-portal infiltration in 3 men who were examined within 3 months of the initial illness.

The exact size of the group which develops unequivocal evidence of chronic hepatitis is not known, but the bulk of evidence suggests that it is small. Barker et al. (161) reported that 5 per cent of one large group of military patients fell in this category 4 months after the onset of hepatitis. Unfortunately, adequate follow-up of these patients was not possible, so that the eventual prognosis could not be determined. In a carefully controlled study, however, Kunkel et al. (157) reported that only 2.3 per cent of 350 young male patients failed to make a complete recovery from acute hepatitis at the end of 1 year.

The progression of acute hepatitis to multiple nodular hyperplasia, post-necrotic cirrhosis, or diffuse portal cirrhosis has been suggested by clinical and epidemiologic observations (134-136, 138, 139, 142, 162). More recently, this concept has been substantiated by information derived from biopsies of the liver in various stages of disease (13, 131, 137). How often viral hepatitis progresses to cirrhosis of the liver is not known, but the available evidence suggests that it occurs infrequently.

The conditions which cause relapse or prolongation and progression of disease are not known exactly, although it has been frequently suggested that premature indulgence in alcohol or excessive activity, and untoward reaction to drugs such as sulfonamides may act as predisposing factors. In addition, the disease is apparently more severe among older age groups and in previously debilitated patients. However, a certain number of patients have relapse in spite of what appears to be ideal therapy. It is not known whether patients with relapse or chronic hepatitis are infectious and have virus in their blood and stool, or whether such occurrences merely represent the physiological inadequacy of the liver damaged by the initial infection.

LABORATORY STUDIES

The Blood. The amount of hemoglobin and the number of erythrocytes are usually normal except in certain patients who have prolonged, chronic, debilitating illness. Such patients may develop secondary anemia. Several observers (134, 149, 163-165) have called attention to the regular pattern of leukocytic response of patients with infectious hepatitis. Characteristically, there is early leukopenia, with both lymphopenia and neutropenia. Subsequently, relative lymphocytosis with the appearance of numerous atypical lymphocytes commonly occurs. These changes are associated with the acute, febrile phase of disease, and a normal relationship between the various cellular components of the blood is found by the end of the 2nd week. The leukocytic changes observed are similar to those found in a number of other acute infectious diseases caused by viruses, i.e., measles (166), dengue (167), sand-fly fever (168), and the suggestion has been made that they represent a characteristic hematologic response to a broad group of infectious diseases (165). Of particular interest is the fact that the atypical lymphocytes found in infectious hepatitis are not to be differentiated from the cells usually considered pathognomonic of infectious mononucleosis.

Coagulation and Bleeding Time. In the small groups of patients in whom the coagulation and bleeding time have been recorded, the determinations have been within normal limits (150). *The fragility of the erythrocytes* is usually normal (150, 169), although in one group of patients slight decrease in fragility was reported during the early icteric phase (170). *The prothrombin time* may be increased in patients with severe disease, but the administration of Vitamin K parenterally causes improvement (156).

The sedimentation rate of erythrocytes is usually not increased early in disease (63, 171-173), and this has been cited as an important differential point in the diagnosis of malaria in which an increased sedimentation rate is present by the 3rd day of disease (174, 175). When jaundice becomes apparent, the rate of sedimentation increases and may persist for many weeks. The fact that the serum globulin is elevated for as long as 3½ months in many patients may offer a partial explanation for the prolonged elevation of the sedimentation rate (176).

Cold Agglutination of Erythrocytes. Tests for cold agglutinins have been performed on a large number of patients in various stages of infectious hepatitis without positive results (177).

Serology. Although no specific diagnostic test has been developed for infectious hepatitis, certain serologic responses have been observed. Eaton et al. (178) and others (179, 180) described a complement fixation test employing as antigens saline extracts of normal liver and liver from patients dying of hepatitis. When tested against serum from patients in various stages of hepatitis, complement fixation was observed in about one-third of the patients. Positive tests were also found in a smaller percentage of normal patients and in patients with atypical pneumonia (178). Olitzki and Bernkopf (181) used similar antigens to demonstrate precipitins in the sera of patients with hepatitis. These tests possibly indicate organ specificity but are unrelated to infectivity, and have little practical value.

Eaton and his group (178) also reported that 34 per cent of a group of patients in the acute phase of hepatitis developed an heterophile antibody which is absorbable on boiled guinea pig kidney and human liver. Other efforts to demonstrate heterophile antibody in large military groups and carefully studied volunteers with hepatitis have been less successful, and in one group of 508 men on whom 2000 tests were performed only 16 (3 per cent) were found to have heterophile antibody in a titer of 1:56. This antibody was absorbed on boiled guinea pig kidney (177). Reports of falsely positive Wassermann and Kahn reactions in as many as 20 per cent of patients with infectious hepatitis have appeared (182, 183). In contrast is a recent survey of 388 known non-luetic patients (177) in which only 1.5 per cent developed positive Kahn tests, and another 2.5 per cent had doubtfully positive tests.

Chlorides. It has been observed that during the acute early icteric phase of disease there is an increase in the interstitial fluid compartment with a tendency to store ingested water. Diminished amounts of chlorides in the plasma and urine are associated with this. During convalescence and restitution of normal hepatic function, diuresis frequently occurs after mobilization of the excess amounts of interstitial fluid. The tendency to store water also diminishes at this time (Labby and Hoagland (184)).

The level of Vitamin A in the plasma is low normal or diminished in proportion to the severity of disease during the acute phase, becoming normal during convalescence. The *lipids* and *phospholipids* are sharply increased during the icteric phase, returning to normal in convalescence (156).

Tests of Hepatic Function. Among the important problems created by the greatly increased incidence of infectious hepatitis have been: (1) the desirability of establishing the diagnosis in the pre-icteric phase or in those patients who fail to develop jaundice; (2) the evaluation of the degree and duration of hepatic damage after convalescence. Because of the lack of a specific serologic test, early laboratory diagnosis depends on the demonstration of evidence of hepatic damage. Such evidence is non-specific, and its value exists largely in certain supportive clinical and epidemiologic data. In order to define early hepatic damage and to determine residual hepatic damage in convalescence, certain groups of laboratory tests have been selected. Considerable experience in evaluating the application of these tests to all stages of infectious hepatitis has resulted

in the definition of a consistently regular pattern of response in the disease (63, 185-188). It is of interest that those tests which are of value in early diagnosis are ordinarily less valuable in determining the degree of recovery. The first test to become positive is usually the bromsulphalein dye retention test (3rd day), and bilirubinuria appears soon thereafter (3rd to 4th day) before the serum bilirubin increases above normal. The cephalin-cholesterol flocculation becomes positive in a significant number of cases on the 4th to 5th day when the immediate direct-reacting serum bilirubin also increases above normal. The total serum bilirubin is increased above normal, on the average, on the 6th to 7th day, and the urine urobilinogen is increased at this time.

By the time jaundice is evident, the flocculation tests, including the cephalin-cholesterol, thymol, and colloidal gold tests, are positive in a large percentage of patients. A sharp depression of cholesterol esters occurs at this time (156). It has been previously noted that bilirubinuria appears before the total serum bilirubin is elevated; the converse is true during recovery, and bilirubinuria disappears before the total serum bilirubin is normal and even while jaundice is still evident (63, 185, 186). The exact explanation of this phenomenon is not known. Attempts to correlate the appearance of bilirubinuria with increased immediate direct-reacting serum bilirubin have not been successful (189).

Jaundice usually disappears and the serum bilirubin becomes normal in the 5th to 6th week, and at this time the bromsulphalein dye retention test ordinarily is normal. The cephalin-cholesterol flocculation and thymol turbidity tests become negative in that order, with significant numbers of patients having negative cephalin flocculations in from 6 to 10 weeks, and negative thymol turbidity tests in 8 to 12 weeks (188). A small number of patients have persistently positive flocculation tests for several months. The exact significance of this is undetermined, although in certain patients attention has been called to the fact that persistent, strong positivity of the thymol turbidity test may precede relapse (156, 190).

Proteins in the Blood. Of considerable interest is the pattern of alteration in serum proteins in infectious hepatitis. Several studies (176, 191-193) have indicated that the characteristic changes consist of an early decline in serum albumin, with an increase in serum globulin. Attention has been called to the fact that these changes are similar to those observed in a number of other acute infectious diseases. These changes are present by the beginning of the 2nd week of disease. The increase in globulin is largely of the gamma globulin fraction. Ordinarily, the albumin becomes normal by the 5th week of disease, although the serum globulin persists at a level higher than normal for as long as $3\frac{1}{2}$ months. The exact significance of this is undetermined, although the possibility must be considered that the increase in globulin may indicate a persistence of antibody (176).

OTHER DATA

Estrogens and Androgens. During the acute phase of hepatitis, young men reveal a significant rise in the urinary excretion of estrogen which is maintained in diminishing amounts until late in convalescence. In such patients, the excre-

ion of 17-ketosteroids is low in the acute phase of disease, with increase to normal in the period from the 3rd to 7th week. Gilder and Hoagland (194) have suggested that in acute hepatitis this need not represent a reciprocal relationship since in all cases the 17-ketosteroids increased in the urine before the estrogen decreased, so that the early depression of the 17-ketosteroids may have been due to the secondary effects of the disease, such as wasting and general debility, rather than to an increase in the circulating estrogens. Of interest in this regard is the observation of gynecomastia occurring during the course of acute hepatitis (195).

Roentgenographic Changes. Pöschl (125) described alterations in the antral portion of the stomach and the first part of the duodenum in patients late in convalescence from acute hepatitis. These changes were considered indicative of gastroduodenitis. In a group of volunteers with experimentally induced infectious hepatitis, Havens et al. (126) reported that 4 out of 11 men developed evidence of gastroduodenitis during the late pre-icteric or early icteric phase. These men had all had normal gastro-intestinal roentgenograms before experimental infection.

Gastroscopic Changes. Knight and Cogswell (127) described changes in the stomach interpreted as superficial gastritis in 7 out of 9 patients during the pre-icteric and early icteric phases of infectious hepatitis. Small aphthous ulcers were observed in the antrum. A similar study made by Havens et al. (126) of 3 volunteers in the pre-icteric or early icteric phase of experimentally induced infectious hepatitis revealed changes in the gastric mucosa of 3 men, compatible with a diagnosis of superficial gastritis. The mucosa was fiery red and edematous, with pools of exudate between the mucosal folds. In 2 of these men, the changes had receded when examined during convalescence, while the third man still had evidence of the earlier changes 7 weeks after the onset of disease. All of these men had normal-appearing gastric mucosa (by gastroscopy) before experimental inoculation. In contrast to these findings are the essentially normal observations of Bank and Dixon (196); however, gastroscopy was performed in most of their patients at least 3 weeks after onset of hepatitis when changes in the gastric mucosa might conceivably have disappeared.

Electrocardiographic Changes. Observations of patients during the acute phase of hepatitis have revealed certain electrocardiographic changes (156, 197, 198). Hoagland and Shank (156) described partial heart block in a small number of patients. The prolongation of the PR interval was at times associated with bradycardia, and both were altered by the administration of atropine. However, in other patients rapid heart rate accompanied the prolongation of PR interval, suggesting that vagotonia was not the predominant mechanism in causing the heart block. Others (198) have described a depression of the T wave during the icteric period, becoming normal during convalescence.

DIFFERENTIAL DIAGNOSIS

Diagnosis in the pre-icteric phase must be made on the basis of epidemiologic and clinical data, since there is no specific serologic test for infectious hepatitis. This also applies to patients in whom jaundice never becomes apparent. The difficulty in early diagnosis is emphasized by the fact that the early constitutional

symptoms associated with high fever in the pre-icteric phase made this disease one of the important causes of "fever of undetermined origin" in the North African theatre during World War II (30). Barker et al. (149) have emphasized the diagnostic importance of tenderness to percussion over the liver early in disease, with posterior cervical adenopathy and splenomegaly. Palpation of the epigastrium frequently causes nausea. Attention has been directed to the leukocytic response of patients in the acute, pre-icteric phase of disease when leukopenia and subsequent relative lymphocytosis with numerous large atypical lymphocytes occur. Evidence of hepatic dysfunction may be suggested early by certain non-specific tests which have been discussed under the section on Tests of Hepatic Function.

During the febrile pre-icteric phase, the diseases to be considered are: *acute bacillary dysentery*, *typhoid* and *paratyphoid fever*, *malaria*, *sand-fly fever*, *dengue*, *pharyngitis with fever*, *acute appendicitis* and *infectious mononucleosis*. When jaundice appears, the following conditions may be considered: *acute* and *subacute cholangitis*, *Weil's disease* and *yellow fever*. Jaundice may also occasionally develop in a variety of acute and chronic infections, as in *malaria*, *brucellosis* and *amebiasis*, occasionally in *pneumococcal pneumonia*, *general septicemias*, *syphilis*, both congenital and acquired (secondary), and not infrequently in *infectious mononucleosis*. The subsequent course of disease, the results of tests of hepatic function, the leukocyte count, the geographic location, and the demonstration of specific etiologic agents or their antibodies make the distinction between these diseases evident. Jaundice occurring during the course of *infectious mononucleosis* may be distinguished by the appearance, after the first week, of a characteristic course with more pronounced lymphadenopathy. Leukocytosis is common in *infectious mononucleosis*, but the atypical lymphocytes customarily thought characteristic of this disease are not to be differentiated from those found in patients with *infectious hepatitis*. A transient and fairly high increase in heterophile antibody (sheep cell agglutination) occurs often in *infectious mononucleosis* at titers of 1:128 to 1:1024 or more. Elevated titers may also occur in a small percentage of patients with *infectious hepatitis*, but they rarely rise above a titer of 1:56, and this heterophile antibody is absorbable on boiled guinea pig kidney and human liver (177, 178).

In addition to the jaundice associated with various infections, other types of jaundice to be distinguished include: (1) *hemolytic*, either congenital or acquired as a result of the effect of various toxic agents; (2) *hepatocellular*, resulting from toxicity of chemicals, notably the halogenated hydrocarbons; cirrhosis of the liver; primary or metastatic carcinoma of the liver; and (3) *obstructive*, due to extra- or intra-obstruction of the biliary tract by calculus or neoplasm. The differential diagnosis of jaundice is particularly difficult in older patients. Careful correlation of the history, clinical findings, results of tests of hepatic function, and often biopsy of the liver are necessary to establish the diagnosis.

TREATMENT

Juvenile Cases. The mildness of the disease in childhood apparently does not necessitate quite as strict a therapeutic regimen as is required in adults. It is

not unusual to find children up and about, making an uneventful convalescence a few days after jaundice has appeared (110). However, the general principles for the care of these patients are similar to those described for adults. Since relapse may occur in juvenile cases, convalescence should be carefully supervised.

Adult Cases. The treatment of the disease is essentially symptomatic and non-specific. None of the chemotherapeutic or antibiotic agents now known has any beneficial effect. Limited therapeutic trials of methionine and choline, and crude liver extract administered intravenously (63, 156, 199-201) have been unsuccessful, although the groups treated were small and further clinical investigation is justified before reaching a definite conclusion. Human gamma globulin has also been of no apparent therapeutic value when given after the onset of disease (63, 202). The two most important therapeutic principles which have been emphasized are the provisions of *bed rest* and *adequate diet* (149, 161, 203, 204). Actually, many of the precepts of therapy were derived from the observation of this disease in troops who had been debilitated by severe physical hardship. It is entirely possible that the therapeutic regimen might be modified in those patients who start their disease in relatively good physical condition. Further evaluation of the effects of rest and diet is necessary.

Early in the course of disease when anorexia, nausea, vomiting or abdominal distress may be severe, it may be necessary to supplement oral intake with fluids administered parenterally in the form of 10 per cent glucose in sterile, distilled water, or 5 per cent glucose in sterile isotonic solution of sodium chloride. A prominent feature of infectious hepatitis is loss of weight, ranging from 10 to 50 pounds, suggesting the necessity of high caloric intake. Recent studies by Hoagland et al. (205) showed that the addition of cream, milk, butter and eggs to the diet makes it possible to increase the calories ingested, replace the weight lost almost from the first day, and shorten the period of convalescence with an earlier return to normal of certain tests of hepatic function. Such a diet should contain 150 grams of protein, 250 grams of carbohydrate, 150 grams of fat high in unsaturated fatty acids of short carbon chains. Supplemental vitamins may be added to the diet, although their absorption or utilization is uncertain. When there is diminution of plasma prothrombin, the parenteral administration of Vitamin K (Hykinone 2.4 mg. ($\frac{1}{8}$ gr.)) daily for 7 days is of benefit. Opiates and barbiturates, particularly the former, are not well tolerated by patients with infectious hepatitis, and should be used with caution. The harmful effects of alcohol have been stressed without adequate evidence.

In convalescence, a full, unrestricted, well-balanced diet containing from 3000 to 4000 calories with at least 150 grams of protein and 400 grams of carbohydrate has been recommended. Emphasis has been placed on the value of a large amount of protein in the diet, although there is suggestive evidence that a well-balanced, high caloric diet, adequate in all components, is equally effective (205, 206). More extensive investigation is necessary to evaluate the comparative effects of various dietary regimens. In the average case, jaundice is gone after the 4th or 5th week. At this time, if the bromsulphalein dye retention test is within normal limits, Barker et al. (149, 161) have recommended that the patient be allowed out of bed, beginning graduated activity according to an exercise

tolerance test. The duration of bed rest necessary has been computed largely from the results of experience with combat troops (149, 161). Further investigation is needed to determine the period of bed rest required for patients in ordinary military or civilian life.

Prognosis. During the acute phase, the immediate prognosis is excellent. However, observation of the records of patients who have died (129) indicates that it is practically impossible to predict a fatal outcome until shortly before death because of the fulminant course which the disease may rarely take. During the acute phase, mental confusion is regarded as a serious prognostic sign. The disease is thought to be more severe in patients over 40 years of age; in those who give a history of hepatitis in the recent past or of exposure to known hepatotoxic agents such as carbon tetrachloride; or in chronic alcoholism. In general, those patients who have severe jaundice are more likely to have a prolonged illness (150). Gauld has reported that the mortality rate increased in American troops in the European and North African epidemics, with a 5-fold increase in the death rate in the year 1944-1945 when compared with the preceding 15 months (31).

Prophylaxis and Control. Epidemiologic observations and experimental studies suggest that the intestinal-oral route may be one of the natural ways of spread of infectious hepatitis. In support of this concept are the following facts: (1) virus is known to be present in the feces of patients in the acute phase of disease, and may be transmitted to man by feeding; (2) infectious hepatitis frequently flourishes in environments where sanitation is poor; (3) water-borne, food-borne and milk-borne epidemics have been reported.

For this reason, procedures which tend to interrupt the intestinal-oral route have been recommended to control the spread of this disease. Suggested measures include the improvement of the general sanitation of camps and institutions, fly abatement, sterilization of food receptacles, the elimination of possibly infected food handlers, and prevention of fecal contamination of food, water and milk supplies. Control of water supply is important, but the degree of chlorination effective against hepatitis virus is not yet determined. Neefe et al. (207) have shown that 30-minute total and free residual chlorine concentrations of 1.1 and 0.4 p.p.m. respectively are apparently effective in inactivating hepatitis virus in water which has been previously coagulated, settled and filtered. Without the preliminary treatment, however, the same 30-minute residual total chlorine concentration (1 p.p.m.) was ineffective (82).

Detection of human carriers or subclinical cases is impossible because of the lack of either a specific immunologic test or a susceptible laboratory animal with which to determine the presence of virus. However, every possible effort should be made, particularly in respect to food handlers. The general control measures for enteric infection should be observed, and the feces and urine should be sterilized. Particular effort should be made to sterilize all needles and syringes which come in contact with the blood of such patients. Moreover, stylets or needles used to obtain blood for blood counts should be sterilized after use on such patients. It is as yet unknown how long virus remains in the stool or blood. Since the limits of infectivity are not determined, it is advisable to regard the stools as

potentially infectious for at least 1 month after the onset of disease, and to advise against such patients' acting as blood donors for at least 1 to 2 years after the onset of disease.

Specifically, normal human gamma globulin (117-119) in a dosage ranging from 0.06 to 0.12 cc. per pound of body weight, administered intramuscularly, is an effective prophylactic when given up to within 6 days before the onset of disease. For the average adult, 10 cc. is sufficient. It is estimated that such passive protection lasts 6 to 8 weeks. Passive immunization is recommended for exposed persons who, by reason of their general condition, are not likely to tolerate another illness; for certain family outbreaks; and to interrupt occasionally the course of epidemics in institutions or camps.

HOMOLOGOUS SERUM HEPATITIS (POST-VACCINAL HEPATITIS;
INOCULATION JAUNDICE; TRANSFUSION JAUNDICE;
LATE ARSPIENAMINE JAUNDICE)

Introduction. Reference has been made to the appellative dilemma into which the nomenclature of viral hepatitis has fallen. The artificial definition, homologous serum hepatitis, has been used to describe arbitrarily a form of hepatitis ordinarily induced by the parenteral inoculation of human blood or its products obtained from a person who, though not apparently ill, is carrying the causative virus in his blood. In this way, tens of thousands of infections have been produced accidentally in persons who have received injections of human convalescent serum, vaccine containing human serum, plasma, and rarely whole blood. During the years of World War II, the simultaneous occurrence of large outbreaks of such artificially induced hepatitis, as well as epidemics of the naturally occurring disease, infectious hepatitis, made it possible to study certain aspects of the relationship between these two forms of disease.

History. In retrospect, it seems likely that the outbreak of jaundice following vaccination with glycerinated humanized lymph (small pox vaccine) described by Lürman (208) in 1885 represented homologous serum hepatitis. In 1920, Stokes et al. (209) and, later, Ruge (210, 211) suggested that the increased incidence of jaundice among syphilitic patients treated with arsenical drugs and bismuth was caused by an infectious agent rather than a chemical. However, it is only within recent years that the concept of the viral etiology of homologous serum hepatitis was evolved. Findlay and his associates (212-214) described the occurrence of jaundice following immunization against yellow fever, and were the first to suggest that the etiologic agent was filtrable and derived from the human serum used to prepare the vaccine. Numerous reports of other similar outbreaks of jaundice associated with immunization with yellow fever vaccine (215-217), pappataci fever vaccine (218), measles convalescent serum (219, 220), pooled mumps convalescent plasma (221, 222), and transfusions of pooled plasma or blood (223-236) have appeared. Records of the U. S. Army epidemic of 1942 revealed 51,337 cases of homologous serum hepatitis following the administration of ieterogenic yellow fever vaccine (101, 237-239). It is now apparent that this form of hepatitis may also be transmitted from patient to patient by improperly

sterilized syringes or needles employed in giving anti-syphilitic therapy (240-249), or penicillin (250-252), or in withdrawing blood (253, 254) for various procedures such as observation of the erythrocyte sedimentation rate or blood counts.

EXPERIMENTS IN TRANSMISSION OF VIRUS TO ANIMALS

As in infectious hepatitis, the virus causing homologous serum hepatitis in man has not been transmitted to laboratory animals. Attempts to transfer the disease to rhesus monkeys, rabbits, mice in the embryonic stage and new-born, cotton rats, pigs, Syrian hamsters, guinea pigs, horses, young chicks, and embryonating hen's eggs and tissue culture have been unsuccessful (239, 255-260).

Several reports have appeared, describing hepatitis in horses following the administration of homologous serum alone or in conjunction with vaccines for the purpose of conveying active or passive immunity against "horse sickness" (261), equine encephalomyelitis (262-264), anthrax (265), and *Clostridium welchi* toxins, (Gordon; cited by Findlay and MacCallum (213)). Whether any relationship exists between the agent causing equine hepatitis and homologous serum hepatitis in man is not known.

EXPERIMENTS IN TRANSMISSION OF VIRUS TO VOLUNTEERS

The available information about homologous serum hepatitis virus has been derived largely from experiments in its transmission to volunteers (Table IV). In 1943, Oliphant et al. (217) reported the transmission of hepatitis to volunteers by the parenteral inoculation of serum obtained from patients in the acute phase of homologous serum hepatitis, resulting from the administration of known icterogenic yellow fever vaccine. These results were corroborated and extended by others (260, 266-271). It was shown that the virus is filtrable through Berkefeld N and Seitz E K filters, and is resistant to temperatures of 56° to 60° C. for at least 30 minutes. It survives at a temperature of 4° C. for a long period (217); at a temperature of -10° to -20° C. for 4½ years, but apparently becoming inactive after 5 years at this temperature (282); in a desiccated state at room temperature for at least a year (267); in serum containing merthiolate in concentration 1:2000 (221), in a mixture of equal parts of phenol and ether in 0.5 per cent concentration (225), and in a 0.2 per cent concentration of tricresol (213, 214). It is inactivated in serum following exposure to ultraviolet light for 1 hour at 2650 Angstrom units (272); heating to 60° C. for 10 hours in human albumin apparently destroys infectivity (283). This virus is transmissible to volunteers in serial passage, and evokes homologous immunity (122, 123).

Attempts to demonstrate virus in the feces of patients in the acute phase of disease have been unsuccessful (86, 268, 273). In addition, the disease has not yet been transmitted experimentally by the oral route with two possible exceptions (255, 260). This latter point may be of importance in the failure to detect virus in the feces, since most of the volunteers employed in these experiments ingested the material to be tested, while only a few received it parenterally.

Limited attempts to detect virus in nasopharyngeal washings and urine have also been unsuccessful, with the possible exception of a single report by Findlay

and Martin (255) who described the occurrence of jaundice in a volunteer 50 days after intranasal inoculation of nasopharyngeal washings from a patient in the acute phase of hepatitis caused by the administration of yellow fever vaccine. This experiment was performed in West Africa where infectious hepatitis was endemic, and it is impossible to be sure that it represents an example of this mode of transmission of disease. The number of experiments performed to date are

TABLE IV

Results of Administration to Volunteers of Materials Obtained from Patients in the Acute Phase of Homologous Serum Jaundice

INOCULUM	AUTHORS	YEAR	ROUTE	NUMBER OF VOLUNTEERS		INCUBATION PERIOD
				Inoculated	Jaundiced	
Feces	Neeffe et al. (86)	1945	O & IG	10	0	days
	Neeffe et al. (86)	1945	P	5	0	
	MacCallum (268)	1945	O	15	0	
	Havens (273)	1946	O	6	0	
Serum	Oliphant et al. (217)	1943	P	186	33	28-133
	Oliphant et al. (217)	1943	P	10	4	120-160
	Oliphant et al. (217)	1943	IN	3	0	
	MacCallum & Bauer (200)	1944	P	16	6	50-127
	MacCallum & Bauer (260)	1944	IN	10	1	80 (30)
	Havens et al. (79, 273)	1944-46	P	13	7	50-71
	Havens et al. (79, 273)	1944-46	O & IN	13	0	
	MacCallum (268)	1945	P	19	12	42-80
	Neeffe et al. (123)	1946	P	25	14	60-135
	Neeffe et al. (123)	1946	O	10	0	
Nasopharyngeal washings	Findlay & Martin (255)	1943	IN	4	1	50
	MacCallum (268)	1945	IN	17	0	
	Neeffe et al. (123)	1946	IN & O	4	0	
Urine	Neeffe et al. (123)	1946	O	1	0	

O = oral; IG = intragastric; P = parenteral; IN = intranasal.

Although the occurrence of non-icteric hepatitis is recognized, only jaundiced cases, in whom the diagnosis could be definite, are recorded here.

insufficient to offer final and conclusive evidence as to whether serum hepatitis virus is in the urine, nasopharyngeal washings, or feces; or whether the disease can be produced by the ingestion of infectious material. It is of interest in this regard that Havens et al. (79, 273) and Neeffe et al. (122, 123) have failed to transmit serum hepatitis to volunteers by the oral inoculation of infectious serum which produced the disease regularly when inoculated parenterally.

It is not known when virus appears in the blood or how long it remains there in homologous serum hepatitis. The accidental contamination of pools of serum

sterilized syringes or needles employed in giving anti-syphilitic therapy (240-249), or penicillin (250-252), or in withdrawing blood (253, 254) for various procedures such as observation of the erythrocyte sedimentation rate or blood counts.

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TABLE IV

Results of Administration to Volunteers of Materials Obtained from Patients in the Acute Phase of Homologous Serum Jaundice

INOCULUM	AUTHORS	YEAR	ROUTE	NUMBER OF VOLUNTEERS		INCUBATION PERIOD
				Inoculated	Jaundiced	
Feces	Neeft et al. (80)	1945	O & IG	19	0	days
	Neeft et al. (86)	1945	P	5	0	
	MacCallum (268)	1945	O	15	0	
	Havens (273)	1946	O	6	0	
Serum	Oliphant et al. (217)	1943	P	186	33	23-133
	Oliphant et al. (217)	1943	P	10	4	120-160
	Oliphant et al. (217)	1943	IN	3	0	
	MacCallum & Bauer (260)	1944	P	16	6	50-127
	MacCallum & Bauer (260)	1944	IN	10	1	80 (36)
	Havens et al. (79, 273)	1944-46	P	13	7	56-71
	Havens et al. (79, 273)	1944-46	O & IN	13	0	
	MacCallum (268)	1945	P	19	12	42-80
	Neeft et al. (123)	1946	P	25	14	60-135
	Neeft et al. (123)	1946	O	10	0	
Nasopharyngeal washings	Findlay & Martin (255)	1943	IN	4	1	50
	MacCallum (268)	1945	IN	17	0	
	Neeft et al. (123)	1946	IN & O	4	0	
Urine	Neeft et al. (123)	1946	O	1	0	

O = oral; IG = intragastric; P = parenteral; IN = intranasal.

Although the occurrence of non-icteric hepatitis is recognized, only jaundiced cases, in whom the diagnosis could be definite, are recorded here.

insufficient to offer final and conclusive evidence as to whether serum hepatitis virus is in the urine, nasopharyngeal washings, or feces; or whether the disease can be produced by the ingestion of infectious material. It is of interest in this regard that Havens et al. (79, 273) and Neeft et al. (122, 123) have failed to transmit serum hepatitis to volunteers by the oral inoculation of infectious serum which produced the disease regularly when inoculated parenterally.

It is not known when virus appears in the blood or how long it remains there in homologous serum hepatitis. The accidental contamination of pools of serum

or plasma by the blood of persons carrying virus while apparently in a state of good health suggests that a carrier state may exist more often than is suspected. The period of infectivity of patients with this disease has been investigated in a limited number of experiments (Table V).

Virus has been detected in the blood of volunteers during the incubation period as well as the acute phase of disease. Neefe et al. (267) recovered virus from the blood 87 days before the onset of hepatitis; Paul et al. (269) detected it 60 days before the appearance of jaundice, and Havens (273) found it 16 days before the appearance of jaundice. Efforts to detect virus in the blood during convalescence have been unsuccessful at intervals of 1 to 5 months after the onset of disease (217, 255, 273). As in infectious hepatitis, it is not known whether a carrier state may exist after recovery, or whether patients with relapse or chronic hepatitis are infectious.

TABLE V

Results of Parenteral Inoculation of Volunteers with Serum of Patients in Various Stages of the Incubation Period and Convalescence of Homologous Serum Jaundice

AUTHORS	YEAR	DAY OF DISEASE SERUM OBTAINED	NUMBER OF VOLUNTEERS	
			Inoculated	Jaundiced
Neefe et al. (267).....	1944	-87	2	2*
Paul et al. (269).....	1945	-60	8	3
Havens (273).....	1946	-16	4	1
Havens (273).....	1946	28 to 32	5	0
MacCallum & Bauer (260).....	1944	66+	5	0
MacCallum & Bauer (260).....	1944	141+	5	0
Oliphant et al. (217).....	1943-44	75 post-jaundice	15	0

* No definite statement of jaundice.

(+) = after appearance of jaundice.

(-) = before onset of disease.

EPIDEMIOLOGY

The geographic distribution of homologous serum hepatitis is not known, but there is evidence to suggest that it is widespread since it has been described in such widely separated areas as the United States (239), Brazil (215), Russia (218), England (213, 219), Sweden (254), and the Middle East (269).

Season. In view of the fact that the present definition of this disease necessitates the concept of artificial inoculation, the seasonal incidence is related entirely to the time of preceding injection of infectious materials. Thus, outbreaks may occur at any time and, up to the present, there is no evidence to suggest that climatic variations affect morbidity rates although it has been suggested that physical hardship may precipitate clinical disease in persons carrying virus (77).

Age. Reports of this form of hepatitis in children have been uncommon (219, 220) but young adults are highly susceptible, as indicated by the experience of recent years. There is suggestive evidence that older age groups are more sus-

ceptible to this disease than to naturally occurring infectious hepatitis. However, the possibility must be considered that many older patients are in a debilitated state of health when they receive potentially large amounts of infectious material in the form of plasma or whole blood.

Incubation Period. The incubation period is long, and is said to range from 4 to 25 weeks, usually being from 40 to 160 days (239). In experimentally induced disease in volunteers, the incubation period ranged from 28 to 160 days.

Mode of Spread of Disease. It has been observed that secondary cases are uncommon among contacts of patients with homologous serum hepatitis, although they do occur (239). It is impossible, at present, to determine whether such secondary cases represent actual contact cases or examples of the naturally occurring epidemic disease. Experimentally, the available evidence suggests

TABLE VI

Results of Attempts to Demonstrate Immunity and Cross Immunity in Volunteers Convalescent from Experimentally Induced Homologous Serum Jaundice

AUTHORS	YEAR	CHALLENGE VIRUS	NUMBER OF CONVALESCENTS			NUMBER OF CONTROLS		
			Inocu- lated	Jaun- diced	Incubation Period days	Inocu- lated	Jaun- diced	Incubation Period days
Oliphant (274)	1944	III*	10	0	—	11	4	85-106
Havens (124)	1945	III	3	3	20-25	11	5	23-31
Neeffe et al. (123)	1946	III	5	4	28-37	6	5	28-32
Neeffe et al. (123)	1946	HSJ**	9	0	—	9	8	60-110

* Infectious hepatitis.

** Homologous serum jaundice

that the parenteral inoculation of human serum containing virus is the most important way of transmission.

Immunity. The capacity of homologous serum hepatitis virus to elicit homologous immunity has been reported by Neeffe et al. (123) who re-inoculated 9 volunteers convalescent 6 to 9 months from experimentally induced homologous serum hepatitis. None of the convalescents became sick, while 8 out of 9 controls contracted serum hepatitis with incubation periods ranging from 60 to 110 days. Both Havens (124) and Neeffe et al. (123) found no evidence of cross immunity between homologous serum hepatitis and infectious hepatitis. Volunteers convalescent from the former disease contracted the latter when re-inoculated with a strain of infectious hepatitis virus. In contrast is the report of Oliphant (274) who described apparently complete protection in 10 volunteers, convalescent from serum hepatitis, when they were re-inoculated with a strain of virus presumed to be infectious hepatitis. This strain of virus was obtained in Italy where infectious hepatitis was epidemic, but it is a matter of interest that it produced hepatitis in the controls after a long incubation period of 85 to 106 days, suggestive of the behavior usually associated with serum hepatitis virus. (Table VI).

Relationship to Infectious Hepatitis. The exact relationship between infectious hepatitis and homologous serum hepatitis is not known, and the artificial definition of the latter form of disease on the basis of probable route of transmission has been confusing. The question has frequently been raised as to whether homologous serum hepatitis may not merely represent the artificial transmission of the naturally occurring disease (275). It is probable that this may occur more frequently than is suspected, but there is no evidence to indicate that this is always the case.

In reviewing the available knowledge about these two forms of hepatitis, it is apparent that certain similarities and differences exist between the two conditions and their etiologic agents. Although these two forms of hepatitis are clinically and pathologically indistinguishable after the onset of disease, attention has been

TABLE VII

*Comparison of Behavior of Viruses of Infectious Hepatitis and Homologous Serum Jaundice in Experimentally Infected Volunteers**

VIRUS	INFECTIOUS HEPATITIS	HOMOLOGOUS SERUM JAUNDICE
1. Filtrable.....	Seitz EK	Seitz EK
2. Resistance to heat.....	56° C. 30 minutes	56° C. 60 minutes
3. Susceptible host.....	Man	Man
4. Incubation period (days).....	15-34	56-134
5. Route of infection (experimental)...	Parenteral or oral inoculation	Parenteral inoculation
6. Virus in stool.....	Acute phase	Not demonstrated
7. Virus in serum.....	Acute phase	Incubation period and acute phase
8. Immunity		
a. Homologous.....	Present	Present
b. Heterologous.....	None apparent	None apparent

* Summarized from the results of Neefe et al. (123) and Havens (16).

directed to the fact that in infectious hepatitis the onset is more apt to be abrupt with fever over 100° F. (37.8° C.) (276). In addition, serum hepatitis may be more severe as it occurs in debilitated patients (277).

The etiologic viruses of both diseases are filtrable, resistant to a temperature of 56° C. for at least 30 minutes, and transmissible to man in serial passage, evoking homologous immunity. Up to the present, they have not been successfully transmitted to laboratory animals or embryonating eggs.

In contrast to these similarities are certain differences which have been consistently reproducible by two different groups of investigators working under the auspices of the Army Epidemiological Board. In Table VII is recorded a comparison of the behavior of two apparently different strains of virus, summarized from the work of Havens (16) and Neefe et al. (123) in experiments with volunteers.

The incubation period of infectious hepatitis is short, ranging from 20 to 40 days, in contrast to the long period (40 to 160 days) of homologous serum hepa-

titis (Fig. 5). Aycock and Oren (275) suggested that the prolonged incubation period of the latter disease is determined by the parenteral inoculation of virus partially neutralized by antibody in the serum. That such a mechanism is not the complete explanation of difference in time interval is suggested by the experimental results of Havens (80, 84, 121, 124, 278) who reported short incubation periods following both parenteral and oral inoculation of volunteers with serum containing the same strain of infectious hepatitis virus. Prolonged incubation periods have indeed been reported in experimentally induced infectious hepatitis when the inoculation was by the parenteral route (77, 78, 272) (Table I). How-

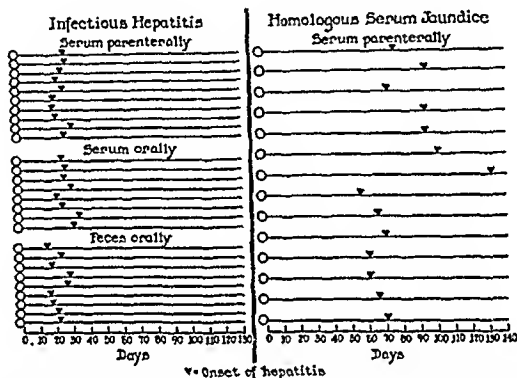


FIG. 5. COMPARISON OF THE DURATION OF INCUBATION PERIODS OF VOLUNTEERS EXPERIMENTALLY INOCULATED WITH A STRAIN OF INFECTIOUS HEPATITIS VIRUS AND A STRAIN OF HOMOLOGOUS SERUM JAUNDICE VIRUS

Each line represents the incubation period and course of disease of a single volunteer. Volunteers were inoculated at 0 days. These strains of hepatitis virus were previously described (Havens, W. P., Jr., et al. (79); Paul, J. R., et al. (269) as a part of experiments conducted by the Neurotropic Virus Disease Commission, U. S. Army).

(Paul, J. R., and Havens, W. P., Jr.: Recent Advances in the Study of Infectious Hepatitis and Serum Jaundice. Trans. Assn. Am. Physicians, 59: 133, 1946.)

(Courtesy of Trans. Assn. Am. Physicians.)

ever, the same inoculums were not tested for infectivity by the oral route, so that no comparisons can be made as to effect of route of inoculation.

The virus of serum jaundice is present in the circulating blood during the long incubation period as well as the active stage of the disease (Fig. 6). Experimentally, it is infectious apparently only when inoculated parenterally, with two possible exceptions. The disease thus produced is not as contagious as infectious hepatitis; evidence of contact infection is rare; and the virus has not been demonstrated in the feces as it has in infectious hepatitis. This fact, in combination with the failure to produce this disease in volunteers by the oral administration of serum known to contain virus, suggests that the intestinal-oral route may not be of importance in its spread, differentiating it in some degree from infectious hepatitis. Of interest in this regard is the comparison of the elimination of two

strains of virus in the feces after parenteral inoculation (278). A strain of infectious hepatitis virus was readily detected in the feces during the acute phase of disease produced by parenteral inoculation. In contrast, a strain of serum hepatitis virus also inoculated parenterally was not found in the feces during the acute disease. In addition, studies on the immunity of volunteers corroborate the epidemiologic experience that patients who have had either infectious hepatitis or homologous serum hepatitis are susceptible when exposed to the other disease (31). Lastly, the long period of viremia in patients with serum hepatitis, in contrast to the much shorter period of viremia in infectious hepatitis, may explain the difference in the prophylactic effect of normal human gamma globulin

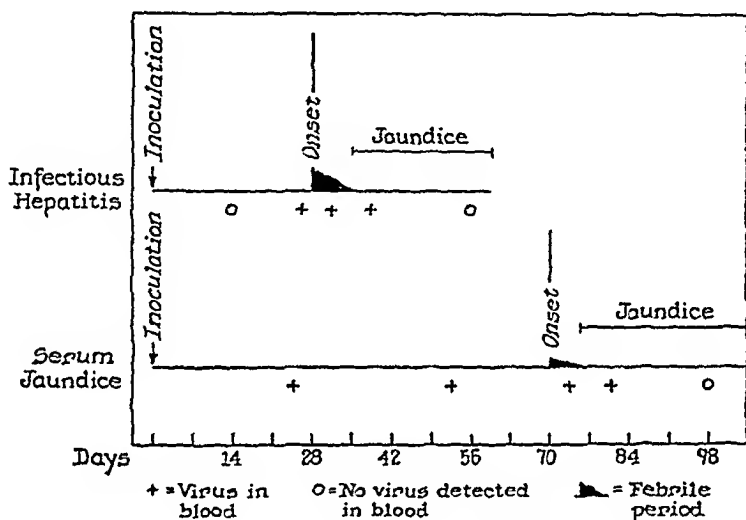


FIG. 6. SCHEMATIC ILLUSTRATION OF RESULTS OF ATTEMPTS TO DEMONSTRATE VIRUS IN THE BLOOD OF PATIENTS DURING THE INCUBATION PERIOD AND COURSE OF DISEASE OF INFECTIOUS HEPATITIS AND HOMOLOGOUS SERUM JAUNDICE

Isolation of virus was determined by the reproduction of disease in volunteers. (Neeffe, J. R., et al. (267); Paul, J. R., et al. (269); Francis, T., Jr., et al. (85); Havens, W. P., Jr. (84, 273)).

(Havens, W. P., Jr., and Paul, J. R.: *Infectious Hepatitis and Serum Hepatitis*. "Viral and Rickettsial Infections of Man," J. B. Lippincott Company, Philadelphia, Pa., 1948.) (Courtesy of the Editor and J. B. Lippincott Company.)

in the two conditions. In the former, questionably favorable results followed the administration of two doses a month apart, while no protection was demonstrable when only one dose was given (279-281). This suggests that the efficacy may have a quantitative relationship with the amount of circulating virus. It is not yet determined whether the differences in route of inoculation, length of incubation period, onset of disease, distribution of virus, period of infectivity, and lack of cross immunity are representative of the activities of actually different viruses or of antigenic differences of various strains of one virus.

Prevention and Control. The long period of asymptomatic viremia during the incubation period, combined with the lack of a specific serologic test or a susceptible laboratory animal to detect virus, makes prevention and control difficult, particularly at present when human plasma and convalescent human serum are

so commonly used. The period of infectivity of patients with this disease is not well defined, and the resistance of the virus is such that there is no easy, practical way to render human blood and its products safe. It has recently been shown, however, that heating human albumin to 60° C. for 10 hours inactivates the virus (283).

In view of these facts and the extreme infectivity of this virus (0.01 cc. of serum is infectious), the following suggestions have been made (230):

(1) Patients with a history of jaundice should not act as blood donors for at least 1 to 2 years after the disappearance of jaundice.

(2) Pools of plasma should be furnished by not more than two donors.

(3) Needles and syringes used in any procedure concerned with drawing blood or parenteral inoculations should be sterilized after each patient.

(4) The need for the use of plasma or convalescent human sera should be carefully considered in the individual patient, with the realization of the danger of possibly transmitting another disease to a patient already sick.

(5) In certain patients, it may be justified to give 10 cc. of gamma globulin intramuscularly on two occasions one month apart, starting a month after plasma transfusions were received. Although the efficacy of gamma globulin is not clearly established, there does seem to be evidence of value in the multiple doses given in the incubation period.

(6) Since hemologous serum hepatitis virus may be inactivated by ultraviolet light (272), the development of a practical method to irradiate plasma or serum may be one solution to the problem.

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THE PHARMACOLOGY, MODE OF ACTION AND THERAPEUTIC POTENTIALITIES OF STILBAMIDINE, PENTAMIDINE, PROPAMIDINE AND OTHER AROMATIC DIAMIDINES—A REVIEW¹

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I. INTRODUCTION

1. *Historical*

During the past decade, the various drugs of the aromatic diamidine series have engaged the interest of several groups of investigators in the field of the experimental chemotherapy of protozoal as well as of bacterial and neoplastic disease. By far the most extensive studies have been conducted at the Liverpool School of Tropical Medicine, where, under the guidance of Warrington Yorke, J. D. Fulton, and their associated groups in various parts of the world, the principle characteristics and clinical value of the diamidines have been established in the treatment of tropical diseases. More recent clinical observations, as well as certain experimental studies, have indicated that the diamidines undoubtedly have a wide biologic application. Since the diamidines are a new type of chemical compound in clinical medicine, it is believed worth while to review their current status. It is hoped thereby that this information may be useful in the study of the many organic compounds containing basic polar groups, such as quaternary nitrogen compounds, nitro-acridines, quinolines, phenazines, phenanthridinium compounds, carbamates, and streptomycin, which are at present engaging the attention of students of the mechanisms of cellular metabolism and of the chemotherapy of disease.

Historically, the introduction of the diamidine compounds and the study of their chemotherapeutic properties are a direct outgrowth of a rather romantic search for agents which would upset the delicate glucose metabolism of trypanosomes. Ehrlich and Gonder (59), Nauss and Yorke (142), Biot (23), Lavarán and Mesnil (121), among others (195) long ago established the fact that the survival time of trypanosomes in blood was prolonged if glucose were added, and that trypanosomes which had become motionless could be revived by the addition of glucose to the media. More recently, Yorke, Adams, and Murgatroyd (195) demonstrated that glucose was an essential metabolite of trypanosomes. *In vitro*, trypanosomes removed relatively enormous amounts of glucose (and oxygen) from any glucose or glucose-serum media. It was natural therefore to seek among the numerous hypoglycaemia-producing agents more satisfactory drugs to replace tryparsamide and germanin, especially since the effects of the latter were believed by the German workers to be the result of hypoglycaemia.

With this concept von Jancsó and von Jancsó (107) began, in 1935, to investi-

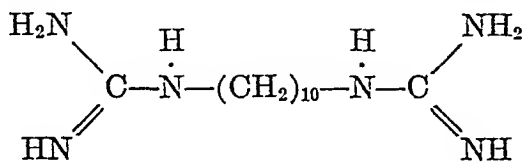
gate the hypoglycaemia-producing guanidine derivatives, such as guanidine hydrochloride and a number of related diguanidines. They found that, as the size of the central inert alkyl chain approached $(CH_2)_{10}$, a striking increase in trypanocidal activity became manifest. Their hypoglycaemia theory was buttressed by the knowledge that the most effective compound, decamethylene diguanidine hydrochloride (Synthalin—see formula which follows) had been used in the pre-insulin era to lower the blood sugar in patients with diabetes mellitus. Synthalin had been banned for clinical usage after Bodo and Marks (27), Dale (47), and others (65, 145) had demonstrated that direct liver damage was the cause of the hypoglycaemia. Nevertheless, the striking experimental effects of synthalin warranted further trial of related drugs (89). It was shown that synthalin was effective in minute doses in mice and rats infected with both arsenic-fast and germanin-fast strains of *T. brucei* and *T. equinum* (154). Schern and Artagaveytia-Allende (153) confirmed the remarkable trypanocidal action of synthalin, and assumed that direct hypoglycaemia *in vivo* thus interfered with trypanosome metabolism. However, in 1937, Yorke and his colleagues (108, 125) showed that the blood sugar level of synthalin-treated animals remained essentially normal unless exceedingly toxic doses of the drug were given. With smaller non-hypoglycaemia-producing doses of this compound, a marked trypanocidal action could be demonstrated. Furthermore, *in vivo* insulin hypoglycaemia was totally ineffective and insulin added *in vitro* did not inhibit trypanosome growth. Thus, despite an erroneous premise, interference with glucose metabolism, a potent new pharmacological agent had been uncovered. Since Yorke believed that a "specific drug action" must have produced these results, he and his co-workers embarked upon a remarkably intensive, systematic series of investigations of related compounds. To him belongs much credit for opening a new important field of biological research.

2. Chemical Considerations and Terminology

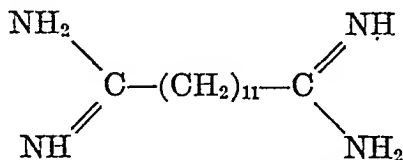
The structural formula of synthalin (decamethylene diguanidine hydrochloride) (see formula which follows) would suggest that a relatively inert alkyl chain acts as a carrier of two biologically active basic guanidine (polar) groups. After synthesizing various diguanidine homologues, King, Lourie, and Yorke (108, 109) and Lourie, and Yorke (125) observed progressive *in vitro* trypanocidal activity with increasing chain length until C_{10} – C_{16} . Increase in the length of the alkyl chain beyond C_{16} was associated with a decrease in activity. The toxicity in mice of these diguanidine homologues changed very little so that the *in vivo* therapeutic index was essentially a function of the *in vitro* activity.

Encouraged by this experience, they undertook next to synthesize homologues of the alkyl diamidine series. These behaved in a similar manner as the carrier chain was increased and the order of effectiveness paralleled that of the diguanidines. N-undecane 1-11 diamidine (compare formulae below) possessed maximal therapeutic activity in the diamidino series. Furthermore, in mice infected with standard laboratory strains of *T. rhodesiense*, *T. brucei*, and *T.*

equiperdum, the diamidines were clearly superior to the diguanidines. A single injection of synthalin 0.075 mg./20 gm. mouse was inferior to 0.025 mg./20 gm. mouse of N-undecane 1-11 diamidine therapeutically in mouse trypanosomiasis. In addition, synthalin was more toxic (32, 109).

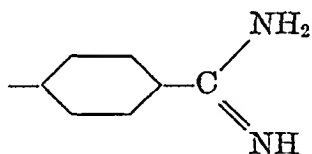


Synthalin
(Decamethylene Diguanidine)



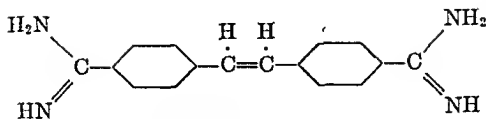
N-undecane 1-11 Diamidine

In a search for less toxic compounds, Dr. H. King of the National Institute for Medical Research (Great Britain), synthesized a large number of guanidines, isothioureas, amidines, and amines with alkyl and alkylene chains (109). These compounds were less effective and more toxic than N-undecane 1-11 diamidine, both *in vitro* and *in vivo*, although many of them displayed significant trypanocidal properties. Following publication of this data by King, Lourie, and Yorke (109) Dr. A. J. Ewins of May and Baker Company instituted a similar investigation into groups of compounds in which aromatic carrier chains were substituted for the alkyl chains (15). He believed that these might offer better possibilities for absorption and excretion under biologic conditions. The aromatic diamidine group was found by Ewins to be the fundamental chemotherapeutic constituent. Any slight change in the radical

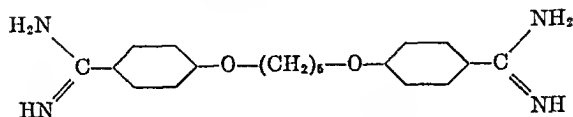


markedly diminished trypanocidal activity. Ewins (127) varied the following factors: (a) length of alkane chain linking the amidino-phenyl residues, (b) substitution and variation of the divalent linkage, (c) change in position of amidine group, and (d) introduction of —O—, —N— and —S— linkages in the alkane chain. From among several hundred compounds screened against several species of trypanosomes as well as against Gram-positive and Gram-negative bacteria, four compounds have been selected as most promising for clinical investigation² (see formulae).

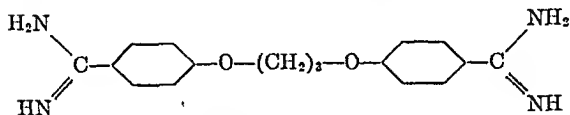
² The terminology of compounds containing the amidine group is somewhat confused. The reader is referred to the discussion on the proper nomenclature for compounds containing the amidine group in Chemical Abstracts, volume 39, number 34, pages 5912-5913, 1945. Thus the name 4,4' stilbenedicarboxamidine is recommended for stilbamidine rather than 4,4' diamidino stilbene. When it is not possible to give a name which treats all amidine groups alike, some are named as substituents with "guanyl" as the prefix for the



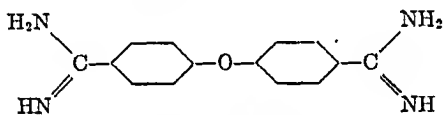
Stilbamidine (M + B 744)



Pentamidine (M + B 800)

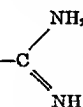


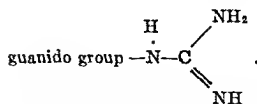
Propamidine (M + B 782)



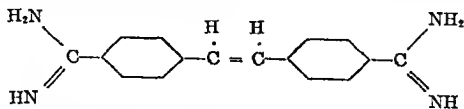
Phenamidine (M + B 736)

Ewins and colleagues (127) have pointed out that these are all symmetrical compounds in which the basic diphenyl residues are linked by a simple alkane chain. One or more of the CH_2 groups of this alkane chain may be replaced

amidine group. The "guanyl" group  should be clearly distinguished from the



According to the classification recommended in Chemical Abstracts the four aromatic compounds would be named as follows:



Trivial name—Stilbamidine

Chemical Abstract—4,4' Stilbenedicarboxamidine

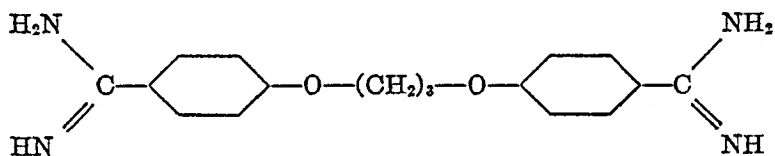
Malaria Survey—SN 251 (191)

by oxygen or may be ethylenic in character. They have concluded that the similar mode of action of these aromatic diamidines was probably related to their similarity of structure. Minor variations in the carrier chain (such as introduction of one or two methyl groups) did not alter the general potency of compounds provided the integrity of the strong terminal basic groups was preserved. These facts are of considerable heuristic significance in any study of the mechanism of anti-biologic action of the diamidines. This review will be confined essentially to the current status of the four aromatic diamidines noted above.

II. MECHANISM OF ACTION OF DIAMIDINES

1. General

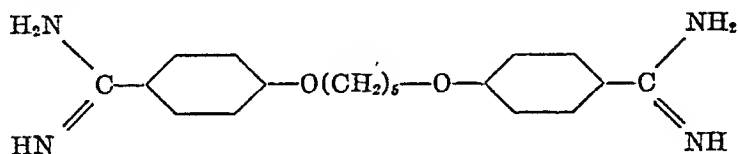
The development of diamidine therapy of Trypanosomiasis was the direct result of a search by pharmacologists for hypoglycaemia-producing agents. Yorke and his colleagues (108, 125) showed definitely that hypoglycaemia was not produced by therapeutically active doses of the diamidines. No direct experimental studies have been reported that would further elucidate the mode



Trivial name—Propamidine

Chemical Abstract—p,p'-(trimethylenedioxy)dibenzamidine

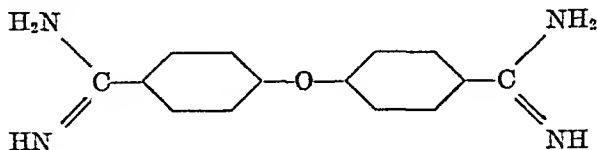
Malaria Survey—SN 6 (191)



Trivial name—Pentamidine

Chemical Abstract—p,p'-(pentamethylenedioxy)dibenzamidine

Malaria Survey—SN 9, 406 (191)



Trivial name—Phenamidine

Chemical Abstract—a) p,p' guanylphenyl ether

or b) bis-(p-guanylphenyl) ether

or c) p,p'-oxydibenzamidine

(c) is considered most correct according to the terminology of chemical abstracts but (b) may be preferable.

Malaria Survey—SN 9, 404 (191)

of action of the diamidines as anti-trypanosomal agents. The wide disparity exhibited by various species of the Genus *Trypanosoma* in their susceptibility to the diamidines remains unexplained.

It is known that stilbamidine-sensitive species (*T. rhodesiense*) are resistant to the inhibition of their oxygen uptake by cyanide (41, 183), whereas stilbamidine-resistant species (*T. lewisi*, *T. cruzi*) are extremely sensitive to such inhibition (83, 30). This apparently basic difference between species susceptibility to both cyanide and stilbamidine would tend to incriminate the important cytochrome enzyme system. Various species of *Leishmania* have been studied which are inhibited by the diamidines but which are as sensitive as *T. cruzi* to inhibition of respiration by cyanide (41, 30). These data merely suggest that the cytochrome-cytochrome oxidase system cannot be used as an indicator for diamidine activity. Further studies of differences in metabolism among closely related species of *Trypanosomes* are necessary to explain diamidine specificity.

Yorke has briefly discussed another important species-difference in metabolism. It is well established that in culture media the utilization of glucose and oxygen by certain species of *Trypanosomes* is relatively enormous. In general, diamidine-sensitive species ("African Group") requirements are ten fold those of the diamidine-insensitive (*T. cruzi*) group (41, 83). Yorke (127) has suggested that a "sugar blockade" is produced by inhibition of the intrinsic aerobic glucose metabolism of the trypanosomes rather than by the production of an environmental hypoglycaemia. Trypanocidal activity would be at a minimum therefore against species (*T. cruzi*) capable of carrying on anaerobic glycolysis easily. This highly speculative theory would at least indicate that the trypanocidal action of diamidines is not that of a "general protoplasmic poison." The more recent observations of Kopae, (v.i.) in which a general denaturation of different nucleoproteins has been observed following exposure to dilute solutions of stilbamidine do not substantiate the latter conclusion. Although present data are scanty, it is probable that two modes of action may be observed, depending on dosage and other variable experimental factors, viz., the action on cells with high aerobic glycolysis, and the toxic action directly on certain proteins which, by virtue of high concentration in specific tissue cells (tumor cells) or by virtue of special configuration, are susceptible to denaturation by diamidines.

Much valuable data have been collected on the antibacterial action of the diamidines. In 1942, Fuller (67) first reported on the action of a series of long chain aliphatic bases, including amines, amidines, guanidines, isothiourreas, and quaternary ammonium compounds against an extensive series of Gram-positive and Gram-negative bacteria. Although great variation in the sensitivity of species was noted, the activity of the long chain aliphatic bases was roughly parallel to the sulfonamides in that the Gram-positive cocci were sensitive to high dilutions whereas the Gram-negative group were generally insensitive. As chain length increased, bactericidal activity increased in a similar manner as in the trypanocidal series (109) reported by Yorke. The drugs were power-

fully bacteriostatic in broth, serum, and exudate in which the sulfonamides are inhibited or inactive.

Shortly thereafter, Thrower and Valentine (175) demonstrated that among the aromatic bases, propamidine possessed the most marked antibacterial activity against Gram-positive cocci in the presence of complex media or even para-aminobenzoic acid. Propamidine activity compared very favorably with penicillin and the sulfonamide drugs but differed in many respects in its mode of action. Stilbamidine was generally somewhat less effective than propamidine. The antibacterial properties of the diamidines were generally similar (61) to those of the sulfonamides, penicillin and gramicidin in that the Gram-positive organisms were more susceptible than the Gram-negative groups. Among these Gram-positive organisms, streptococci were most susceptible whereas in the Gram-negative group the greatest activity was demonstrated against *E. typhosa* and *E. coli* (61). Drug potency was increased three or four fold by raising the pH of the media. Utilizing a synthetic medium Kohn (117) showed that the addition of 1 per cent proteose peptone tripled the amount of propamidine necessary to inhibit *E. coli*. This effect of peptone on propamidine activity was approximately one-third that commonly seen with the sulfonamide drugs. Kohn stated that propamidine acts by inhibiting the catabolic (oxidative) metabolism of bacteria. This is noteworthy since the oxidative metabolism of bacteria is not usually affected by the minimal concentrations in which the sulfonamide derivatives effectively inhibit bacterial growth. Using a different technique, Bernheim (18) added further support to this view. With strains of *E. coli*, *S. aureus*, and *S. albus* in the Warburg apparatus, he investigated the inhibition of oxygen uptake. He employed nephelometry to measure the effect on growth. M/80,000 concentrations of propamidine (and slightly higher concentrations of stilbamidine) inhibited the oxygen uptake of washed bacteria at pH 7.8 with substrates containing nitrogenous constituents (meat extract, peptone). It appeared that in dilute solutions the oxidation of amino acids rather than of carbohydrates was impaired. The oxidation of carbohydrate substrates (pyruvate, glucose) was inhibited only at higher concentrations of the drug, M/9,000. The latter concentration was twenty times smaller, however, than the concentrations (0.1 per cent) employed clinically in the treatment of war wounds. A latent period was present before inhibition of growth or oxygen uptake became apparent and maximum inhibition was not attained for several hours. This latent period was not present if the organisms were shaken with the propamidine before the substrates were added.

The theories of Kohn and Bernheim are also supported by the observation that concentrations as low as M/100,000 (much smaller than those inhibiting oxidation of carbohydrate substrates by bacteria) are lethal to trypanosomes *in vitro*. In a further study, Bernheim (19) presented additional evidence in this direction. The oxidation of L-proline and alanine by *E. coli* was inhibited by propamidine at concentrations which, under the same conditions, accelerated the oxidation of serine and asparagine. The oxidation of the latter two amino acids was inhibited by higher concentrations of propamidine. Bernheim again

noted that the oxidation of glucose, pyruvic acid, and succinate was not affected by concentrations of propamidine which inhibit or accelerate the oxidation of the above amino acids, thus "indicating that the cytochrome system is not involved". That the cytochrome system is not necessarily involved could have been inferred by the fact noted above that diamidine resistant species of trypanosomes (*T. cruzi*, *T. lewisi*) are precisely those which have the most active cytochrome systems as evidenced by their susceptibility to inhibition by cyanide, in contrast to the cyanide resistant species (*T. rhodesiense*) against which the diamidines possess marked therapeutic activity.

Other investigators have hoped to throw some light on the mode of action of the diamidines through the study of diamidine inhibitors. In addition to the effect of peptone described above, it is known that many nitrogen-containing compounds interfere with diamidine bacteriostasis. Snell (172) has demonstrated that certain polyamines permit growth of *L. casei* which are ordinarily inhibited by 0.7 mg. propamidine/10 cc. media, and of *S. lactis* which are inhibited by 0.1 mg./10 cc. media. The polyamines, spermine and spermidine were found to be bactericidal (21). No inhibition of the diamidines was observed at concentrations of these polyamines which were not bactericidal per se. It is interesting to note that the bactericidal activity of spermine and spermidine could be inhibited by nucleic acid (21). The synthetic polyamines, diethylene triamine, triethylene tetra-amidine and tetrathylene penta-amidine were active inhibitors of diamidine bacteriostasis at high concentration (0.1-0.5 Molar) (21).

Phospholipids in the form of soya lecithin or egg lecithin, (60, 61) interfere with propamidine bacteriostasis. Bichowsky (Bichowsky-Slomnitzki) (20, 21, 22) studied both stilbamidine and the somewhat more effective pentamidine action on *E. coli* and *S. aureus*. The presence of peptones, meat extract, Bovril, and Marmite inhibited bacteriostasis but 10% serum did not. Substances with polar groups similar to the diamidines (creatine, creatinine, arginine, guanine) lacked antidiamidine activity. An extensive series of amino acids tested were without activity. In contrast, however, both yeast sodium nucleate (ribose nucleic acid) and animal sodium nucleate (desoxyribose nucleic acid) permitted growth in the presence of the diamidines. This inhibition was observed even when the nucleic acid was added 24 hours after the diamidine. A solution of 0.05% sodium nucleate inhibited bacteriostasis of *E. coli* in high concentrations of stilbamidine (M/25,000). The lethal action of stilbamidine in a concentration of 1:300,000 on *T. vaginalis* was similarly blocked by sodium nucleate. In cultures of *Leishmania* a 1:50,000 stilbamidine solution was completely inhibited by 1% sodium nucleate. The separate purine, pyrimidine and nucleoside constituents of nucleic acids as well as some purine derivatives (uracil, xanthine, hypoxanthine) and the various vitamins of the B complex group³ had no antidiamidine activity. The nucleotides, adenylic and guanylic acids inhibited diamidine bacteriostasis but were $\frac{1}{16}$ to $\frac{1}{256}$ as active as the polynucleotides (21).

³ Thiamine, riboflavin, niacin, choline, pimelic acid, calcium pantothenate, pyridoxine, para-aminobenzoic acid and inositol.

From these data Bichowsky (22) has inferred that in bacteria and protozoa the diamidines tend to concentrate primarily within bodies rich in nucleic acid. The diamidines, as basic substances, may combine with nucleic acids or the nucleoproteins and thus interfere with the utilization of an "essential metabolite" or enzymatic pathway.

This theory is not unlike that proposed for the lethal action on bacteria of various cationic detergents (56, 179, 185). According to the competitive cationic exchange theory, the activity of propamidine, as well as other cationic detergents and basic dyes, depends upon the amount of free toxic diamidine cation available to compete with the non-toxic hydrogen ion for the same anionic position on the (bacterial) cell. It would be expected, therefore, that any increase in the local concentration of competitive non-toxic cations would inhibit diamidine activity. Any decrease in hydrogen ions would thus permit increased adsorption of the diamidine onto the anionic substrate. This would account for the greater antibacterial activity observed by Elson (61) with progressive increase of pH. Increased anions, in the form of phosphate, have been noted to decrease the activity of propamidine (61) at all levels of pH. This is probably due to the formation of a weakly dissociated diamidine salt. The antagonism of the phospholipids of the lecithin type, and of the polyamines has been explained on the basis of their behaviour as non-toxic cations, the former competing with propamidine cation by virtue of the quaternary nitrogen group in the choline residue; the latter because of the presence of free amino groups (63). This theory of cationic competition does not explain the lack of inhibition by many small-molecular compounds containing free cations. The inactivation of propamidine by nucleic acid has been offered as evidence that the nucleic acid anions combine with the propamidine cation, and are in fact the critical point at which the nucleoproteins of the cell are altered. Using entirely different physical methods for the study of tumor cell nucleoproteins, Kopac (118, 119, 120) has reached an essentially similar conclusion, namely that nucleoproteins are, to a greater or lesser extent, susceptible to physicochemical alteration by stilbamidine.

2. Tumor Inhibition

Sir Henry Dale was the first to suspect that the remarkable inhibitory effects of the diamidines on the oxidative metabolism of bacteria and protozoa might extend across to neoplastic and normal animal tissue. At his suggestion, Dickens in 1939 (55) studied the inhibition of oxidative metabolism by the alkyl diamidines. Inhibition of the Pasteur effect on animal tissue had been previously known to be manifested by quaternary Nitrogen and related compounds, such as the acridines, quinolines, and phenazine derivatives all containing active basic substituents. After studying the effect on rat brain, Jensen Sarcoma, and yeast, Dickens concluded that N-undecane 1-11 diamidine and synthalin were "the most powerful inhibitors" of the Pasteur effect yet described.

The *Pasteur effect* is a term used to designate the inhibition of cellular glycolysis when oxygen is present. Glycolysis represents the biologic formation of two

moles of l-lactic acid from one mole of hexose or its glycogen equivalent. Glycolysis is an anaerobic dismutation and is not an essential prerequisite for carbohydrate oxidation. Malignant tissues and leukemic cells show only a slight Pasteur effect.

Bernheim (18) independently noted that the oxygen uptake of suspensions of rat kidney and liver was not inhibited by M/8000 propamidine whereas rat brain oxidation of glucose was inhibited 50% by concentrations of M/30,000. Pyruvate oxidation was inhibited by concentrations of M/15,000. Whether these discrepancies were due to better penetration of brain tissue slices or were a manifestation of the protective action of nucleic acid and nucleoprotein present in rat liver or kidney was not clear.

More recently, Haddow, Harris, Kon, and Roe (92) in a series of studies designed to define the molecular conditions for the growth-inhibitory action of drugs in transplanted animal tumors, examined a group of basically substituted stilbenes. In rats treated with 4,4' dimethylaminostilbene in doses of 5 mg./100 gm. body weight, a definite retardation of body growth as well as tumor growth (Walker Sarcoma) was produced. This was associated with histological and cytological alterations ascribed to the drug. Unfortunately, these effects could be elicited only within narrow limits because of the marked toxicity of the drug. Nevertheless, to a lesser extent, the same effects were noted in mice suffering from Crocker Sarcoma, C63 carcinoma, and spontaneous tumors. These authors could not ascribe the action to the deprivation of essential sulfur-containing amino acids, since they were unable to demonstrate increased excretion of mercapturic acid or neutral sulphur. They believe that the action on growth is the result of a "more direct, and perhaps competitive disturbance in cell metabolism."

Kopac (118) also has confirmed the inhibitory action of physiological dilutions of the diamidines on tumor cells in tissue culture media, and has made more detailed study (119, 120) on the rate of denaturation of several nucleoproteins, (liver, tobacco mosaic virus, protamine-nucleate, mitochondria, cytoplasmic fractions), exposed to one or more diamidines or other oncolytic agents. The drugs were observed at physiologically effective concentrations either alone or in combination. By use of physical methods, it was possible to divide compounds into several groups according to their ability either to enhance or inhibit interfacial denaturation of proteins. Denaturation was measured by a semi-automatic drop retraction apparatus in which the protein is simultaneously exposed to an oil-water interface as well as to one or more chemical agents. In this manner a dual augmentation of denaturing agents may be studied. By means of this rather complex technique certain compounds were found to augment surface forces. According to Kopac, these compounds act by "weakening critical side-chain linkages" with the production of two dimensional instead of three dimensional protein patterns. Among the substances studied, stilbamidino and the chloroethyl amines (nitrogen mustards) were the most potent denaturing agents. Replacement of the stilbene linkage with oxygen which markedly alters the absorption spectra, fluorescence and other properties, as in pentami-

dine, propamidine, and phenamidine, resulted in decreased denaturation by a "strengthening of the critical side chain linkages." Furthermore, previous treatment of liver nucleoprotein with any of the phenoxy-diamidines blocked the striking denaturation observed with stilbamidine (stilbene linkage). No adequate explanation for these observations has been offered. It is apparent that stilbamidine differs from the phenoxy compounds not only in its physical and chemical properties but also in its toxic manifestations.

Snapper (167) and Snapper and Schneid (167, 170, 171) have recently added further evidence in favor of protein denaturation by stilbamidine. Within several weeks after a course of treatment with stilbamidine, seven of nine patients treated with stilbamidine for Multiple Myeloma developed basophilic inclusion granules in 80% or more of their myeloma cells. These granules disappeared after application of the enzyme, ribonuclease, to the cells. By means of ultraviolet-spectrophotometry and ultraviolet microscopy the specific absorption spectra for nucleic acid was observed in the granules (171). The evidence for the presence of stilbamidine was somewhat less convincing despite the widely separated absorption bands of the two substances in question. From this evidence, and from Kopac's work demonstrating that stilbamidine dissociates protamine ribonucleate, Snapper concluded that stilbamidine interferes with the metabolism of the myeloma cells. It is of interest that in a large series of patients, Snapper (169, 170) observed the stilbamidine ribosenucleic acid precipitates only in patients with high blood levels of the globulin peculiar to myeloma. He pointed out that the myeloma cells are unique among neoplastic cell-types by their production of large amounts of a specific protein, which, by virtue of its constitution or its abundance in certain cells, may be susceptible to stilbamidine denaturation. If subsequent studies of the granules confirm the presence of true stilbamidine-nucleic acid complexes, this will have been the first instance of the production of specific morphological alterations by a parenterally administered chemical on human neoplastic cells.

Several investigators agree that stilbamidine causes an alteration in the interfacial forces acting on protein surfaces. This implies fundamental alteration of nucleoproteins either by (1) irreversible dissociation of nucleic acid from protein or (2) blockage of critical intramolecular linkages (119, 120, 171). Since it is well established that the development of many different neoplasms is associated with marked increase in both desoxyribose nucleic acid (chromosomes) and ribose nucleic acid (cytoplasm), the mode of action of the diamidines on nucleoproteins may well possess a significant bearing on the nucleic acid equilibrium in carcinogenesis. Surely the increase in both trypanocidal and bactericidal activity up to but not beyond an optimum chain length of C_{10-14} suggests that surface-acting properties may be responsible for therapeutic action by denaturation of some essential protein constituent. On the other hand, the wide variation in diamidine action on closely related species of trypanosomes, and the effectiveness of both stilbene and phenoxydiamidines in protozoal infections, despite the latter's relative inability to produce protein denaturation, point to the fact that at least two mechanisms are probably involved, namely

the inhibition of certain specific, as yet undetermined, enzyme systems, and the general alteration of nucleoproteins which may, in fact, play a greater or lesser rôle in the enzyme systems of different species of bacterial, protozoal, and tumor cells.

III. PHARMACOLOGY

1. *Chemical and Physical Characteristics*

4,4' diamidino-stilbene (stilbamidine, M & B 744) is a white crystalline powder. It can be heated in solution to 70–80°C. for a short time (15 minutes) without decomposition. The solid is stable in the dark. A melting point cannot be determined as stilbamidine turns yellow at 200°C. and decomposes at 290°C. The compound is soluble in water up to 40 grams in 100 ml. at 25°C. It is fairly soluble in glucose solutions and moderately soluble in the usual blood-citrate mixtures (0.08 mg./ml.). It is precipitated by stronger solutions of citrate, oxalate, strong acids, saline solutions (especially in the cold), and almost all common protein precipitants with the exception of dialyzed iron (54), and basic lead acetate (73). Pentamidine and propamidine are more soluble in these solutions, but their solubility characteristics are in general similar to stilbamidine. When stilbamidine is added to human or mouse sera in concentrations greater than 0.5%, it precipitates out as a base which is insoluble in water but is soluble in dilute hydrochloric acid (73). This fact may have some bearing upon the immediate toxic reactions observed in man following injections of concentrated solutions (v.i.). During the past several years, the diisethionate salt, containing only 60% of the base present in the hydrochloride salt, has been used for clinical and experimental purposes because of its greater solubility and better tolerance during intravenous injections. Maximum tolerated doses of the diisethionate (hydroxyethyl sulphonate) are from 50% to 100% greater than of the hydrochloride.

After standing for several months stilbamidine powder turns pale yellow, but this is not associated with any significant alteration in the absorption spectra, toxicity or therapeutic action (74). In contrast there is ample evidence (16, 70, 79, 100), to prove that solutions of unsaturated stilbenes (stilbamidine, monomethyl stilbamidine) will be markedly affected by even fifteen minutes of sunlight, whereas similar solutions kept in total darkness over a period of many months remained unaltered. Fulton and Yorke (79) have shown marked increase in toxicity and decrease in therapeutic activity in both man and animals after injections of solutions exposed to sunlight. This deterioration is due to the action of ultraviolet light on the unsaturated stilbene linkage. The compounds, 4,4' diamidino α,α' dimethyl stilbene, pentamidine, and propamidine are unaffected by light. According to Fulton (70), the principle chemical change is a saturation of the ethylenic linkage by direct hydration with the production of a colorless compound, 4,4' diamidino phenyl benzyl carbinol. Barber, Slack and Wien (16), concur in this interpretation. On the other hand, Henry (100, 101, 102) states that sunlight acts by accelerating a process of cis-

trans isomerization and the amidine groups, which are unstable, are converted to amido groups. 4,4' diamidino phenyl benzyl carbinol was not the principal product of irradiation. Hydrolysis of one or both amidine groups was associated with the liberation of ammonia. The formation of 4-amido: 4' amidinostilbene monohydrochloride (most probably the trans form) and 4,4' diamido-stilbene was confirmed by isolation. Experimental evidence suggested that the principle product produced by irradiation of stilbamidine solutions was a dimer of stilbamidine. This dimer would be 1,2,3,4-tetra (4' amidinophenyl) cyclobutane. By comparative studies of spectrographic absorption curves, Goodwin (87), confirmed that saturation of the ethylenic linkage parallels the alterations in toxicity and therapeutic efficacy produced by exposure of the various unsaturated compounds to light. No degradation or loss of efficiency occurred if solutions were placed in the dark for long periods of time (79). Boiling for 2 minutes or heating to 60°C. for five minutes did not alter the properties of these solutions. Devine (54), observed that alteration in pH did not substantially affect the rate of degradation in sunlight. The observations of Henry (100), differ from those noted more recently by Fulton and Goodwin (75). They studied cis and trans stilbamidine both therapeutically and spectrographically after irradiation with ultraviolet light. These investigators could find no evidence of ammonium chloride formation. Their observations supported the concept that cis-trans isomerization occurred. In dilute solutions (0.05%) the cis form was converted to the trans form but no saturation of the linkage occurred. In 0.2% solutions of cis stilbamidine irradiation produced the trans form and also saturation of the ethylenic linkage in the trans compound. There was no evidence for trans-cis formation or splitting of the double bond. The trans stilbamidine was therapeutically active. The cis compound was more toxic and less effective therapeutically.

Of the various members of the diamidine series, stilbamidine (and other unsaturated stilbenes) alone manifest a remarkably brilliant blue fluorescence even in extremely dilute solutions (1 part in 1000 million) when exposed to ultraviolet light (103). In addition, it possesses a strong narrow spectral absorption band detectable in concentrations as low as 1.0 microgram per cubic centimeter. Since maximum absorption characteristically is obtained at 329 m μ (103), little interference may be expected from most biological substances.⁴ Maximum fluorescence occurs under monochromatic light of 310 m μ (103). The cis isomer does not fluoresce when exposed to ultraviolet light (101, 102). These properties should make it feasible to determine stilbamidine distribution in certain biological fluids by spectrophotometric or fluorometric methods. Despite these characteristics, a convenient accurate method for quantitative determination of stilbamidine has not yet been developed. The difficulty in quantitative estimation is due to the marked adsorption of stilbamidine on proteins and other compounds of large molecular weight or surface area. For example, stilbamidine is almost quantitatively adsorbed to cellulose, Kaolin, Fuller's earth, charcoal, filter

⁴ Cis Stilbamidine-Molecular extinction (E) shows a maximum 14,000 at λ 299 m μ .

Trans-Stilbamidine-Molecular extinction (E) shows a maximum 37,800 at λ 329 m μ . (75).

paper, beef protein, and the proteins of serum, plasma and other body fluids (54, 73, 103). In urine stilbamidine can usually be detected without laborious separation from biological substances which obscure or quench fluorescence and interfere with the absorption spectra (103). Because of the marked adsorptive capacities several workers have suggested the possibility that methods of assay involving adsorption on various chromatographic columns followed by elution with certain solvents, and finally either spectrophotometric or fluorometric determination might be used. Despite numerous modifications, only the blood levels obtained in man immediately after maximum tolerated doses administered intravenously can be measured with reasonable accuracy.

2. Methods of Determination

The properties of marked adsorption, insolubility, and fluorescence, together with the specific absorption spectra have been employed in order to develop quantitative methods of determination. Such techniques are necessary before study of the absorption, excretion, and distribution of stilbamidine in the body of man and animals can be initiated.

By gross examination of the organs of mice for fluorescence, Hawking and Smiles (99), concluded that stilbamidine was found principally in the liver and kidneys, and to a lesser extent in the small intestine and skin. It was not possible to determine how much quenching was produced by the varying concentrations of blood and other proteins in the various organs. Gross examination of organs or biological fluids, can supply only limited information. It is too crude a method for any type of quantitative assay.

With the ultraviolet fluorescent microscope, marked rapid adsorption of stilbamidine by sensitive species of trypanosomes was seen by Hawking and Smiles (99) both *in vitro* and *in vivo*. Most of the drug was deposited in the blepharoplast and anterior cytoplasmic granules of living flagellates. Within ten minutes after intraperitoneal injection, these authors noted a partition ratio between trypanosome and plasma layers of approximately 4,400. *In vitro* partition ratios of 1,400 were observed (98). The marked therapeutic action of minute doses of stilbamidine would seem to be a reflection of its selective adsorption by certain chemical components of the viable protozoal organism, since it was taken up only by live trypanosomes and at body temperatures (98). A modification of this technique has been used by Snapper (106) and Snapper et al. (171), who demonstrated that myeloma cells contain precipitated stilbamidine-nucleic acid complexes. The specific absorption spectrum and the fluorescence of stilbamidine may thus be utilized in a photographic study of microscopic structures to indicate the site of stilbamidine adsorption.

Similarly, marked adsorption to filter paper is the basis of a simple chromatographic technique for studying stilbamidine excretion in urine. By spotting out a standard sized drop of urine on filter paper, it is possible to detect (103) concentrations of 0.0005 per cent under a simple Wood's light. Moreover, such filter paper may be washed repeatedly, dried, and stored without any loss of fluorescence. Although this method may be applied to serum, plasma, cerebro-

spinal fluid, and even milk, the margin of error at the concentrations of stilbamidine tolerated physiologically is from 50 to 100%. Moreover, careful observers encounter an error of 29% with this method even when applied to urine studies. It appears to be a quick simple means of determining whether any particular patient is excreting significant quantities of stilbamidine in the urine. Henry and Grindley (103) have modified this technique for urine assay by adsorbing stilbamidine on cellulose pulp and examining for fluorescence. Subsequently the pulp was subjected to Kjeldahl analysis or Nesslerized for the determination of stilbamidine nitrogen. In the presence of other types of adsorbable nitrogen (albuminuria), this method is wholly unsuitable.

In the state of equilibrium maintained by the buffers existing in whole blood and other biological fluids, stilbamidine has been assumed to be adsorbed on the blood proteins as a relatively nonionized base. Nevertheless, it has been found possible to remove most of the drug by percolation through canisters containing ion exchange columns.

By increasing the hydrogen ion concentration, several investigators have demonstrated both partial reversibility of stilbamidine adsorption to trypanosomes (98, 99), as well as complete reversibility of adsorption to animal proteins and cellulose products. Guided by these principles, Henry and Grindley (103) devised a method for quantitative determination of stilbamidine consisting of (a) adsorption of stilbamidine on a column of cellulose, (b) elution with dilute HCl, and (c) quantitative spectrophotometric determination of the drug from the eluate. With this technique recovery levels of from 80% to 90% were possible in relatively concentrated solutions of stilbamidine in blood. With the levels obtained following parenteral injections in man and animals the recovery rate was much lower. Saltzman (150), has recently claimed somewhat better recoveries from urine and blood which have been percolated through a "Decalso" column, eluted with an HCl-alcohol mixture and measured with a fluorophotometer. Reproducible recovery of stilbamidine from plasma mixtures as dilute as 9 to 30 gamma per cc. were stated to be easily attainable, and in urine concentrations as low as a 3 gamma per cc. could be measured without the necessity for preliminary protein precipitation.

Fulton and Goodwin (73) have found, however, that it is necessary to remove proteins from the serum. For this purpose they have used dialyzed iron which was found not to precipitate stilbamidine. Satisfactory spectrophotometric readings were obtainable with levels of 2.5 gamma/cc. and higher in sera of mice treated with stilbamidine by various routes of administration. Unfortunately, serum levels of stilbamidine fall rapidly below this level after the first hour of treatment. Therapeutic activity probably persists well below levels detectable with any of the present methods of determination.

A colorimetric method for determination of stilbamidine has been devised by Devine (54) based upon the combination of aromatic diamidines and glyoxal in warm alkaline aqueous solution with the production of a yellow glyoxalidone compound. The color of the latter is intensified subsequently by the addition of acid. The depth of color is determined in a Hilger "Spekker" absorptiometer.

Slight changes in technique, however, alter greatly the development of color. Accordingly, Fuller (68) has tried to stabilize the reaction by heating in a borate buffer. Although this method was sensitive, the reaction was common to all the unsubstituted aromatic amidines. Certain substances in biological fluids yielded false color reactions. Furthermore, the reaction was technically difficult, since an excess of glyoxal inhibited the development of the colored compound (68). Jackson, Kuhl and Irvin (106), have utilized the reaction of aromatic amidines with glyoxal and benzaldehyde to form a fluorescent 2-aryl, 4-benzal, 5-glyoxalidone compound in alkaline aqueous solutions. This fluorometric method provided a better relationship between the intensity of fluorescence and the concentration of the amidine and was less dependent upon the concentration of reagents. It was generally applicable to the aromatic diamidines whereas Fuller's method was inapplicable to propamidine. Aliphatic amidines, guanidine, arginine, creatine, creatinine, urea, ammonia and uric acid were stated not to interfere with the reproducibility of results. Protein adsorption was responsible for only a small loss when dialyzed iron was used as a protein precipitant. The technique permitted determinations on concentrations as low as 80 gamma/100 ml. with a 4-8% error. With butanol extraction reproducible results were obtainable at 50 gamma/100 ml. This method may suffer from a lack of sufficient accuracy and sensitivity at the low blood levels which occur in from 30 to 60 minutes after a given injection of diamidine. Consequently an adequate understanding of the adsorption of diamidines onto various tissue proteins, and their distribution and excretion must await the development of more satisfactory methods of assay.

3. Absorption, Distribution and Excretion in the Body

Within a few minutes after its administration by either oral, subcutaneous, intramuscular or intravenous routes, stilbamidine may be detected in relatively high concentrations in the blood and the urine of man or various experimental animals (73, 103, 188). After a single intravenous or intramuscular injection of a maximally tolerated dose, Wien (188) and Fulton and Goodwin (73) observed peak blood levels of 30 to 40 $\mu\text{g}/\text{cc}$. These quickly fell within 30 minutes despite the differences in the maximum doses attainable via the various routes of administration. After two hours less than 2 $\mu\text{g}/\text{cc}$. were detectable (73). It was notable that, even after large oral doses, peak levels were maintained for only a short period of time. In mice (73), the subcutaneous route afforded slight prolongation of the high blood levels. This may be related to the somewhat higher therapeutic indices (80) observed when trypanosome infections were treated by this route. These higher therapeutic indices may also reflect activity at low blood levels, undetectable with the present methods of determination, which persist after a single injection of stilbamidine (73, 103). According to Fulton (71), such levels (less than 2 $\mu\text{g}/\text{cc}$.) are capable of definite therapeutic and prophylactic activity in mice. Therapeutic indices in mice correlate roughly with the overall duration of high blood levels (73, 80), being smallest when oral or intravenous routes of administration are employed. Just

of synthalin as well as of certain alkyl diamidines was demonstrable in minute doses which did not significantly alter the blood glucose level. They ascribed the therapeutic property to a specific action of the basic diamidine groups. In overwhelming toxic doses, synthalin (decamethylene diguanidine) (26), and certain alkylene diamidines (31), had been previously observed to produce hypoglycaemia associated with a loss of liver glucose and glycogen together with gross evidences of renal and hepatic damage. According to Bodo and Marks (27), the mechanism of synthalin hypoglycaemia differed significantly from that of insulin. For example, glucose freed as a result of the glycogenolytic action of synthalin on the liver was not utilized or converted to muscle glycogen. After synthalin had been administered the hyperglycaemic response to adrenalin was abolished, probably as a result of glycogen depletion of the liver. Broom (31), in a comparative study of various diguanidines and diamidines, noted that the latter did not affect carbohydrate metabolism as readily as the former compounds, but nevertheless produced marked changes when administered in large doses. In rabbits a half-minimal lethal intravenous dose (5 mg./kg.) of N-undecane 1-11 diamidine, an alkyl analogue of stilbamidine, was noted by Devine (52), to produce a rapid rise of blood urea of about 30 mg. %, but the blood sugar was unaffected. However, maximum tolerated doses (10 mg./kg.) produced transient hyperglycaemia associated with marked nitrogen retention, albuminuria and casts. Lethal doses produced fatal hypoglycaemia with or without an initial hyperglycaemia, probably depending upon the hepatic reserves prior to the experiment. Damage to the liver and its deaminizing function seemed to parallel the degree of glycogenolysis. In two patients given 150 mg. of N-undecane 1-11 diamidine per day orally for three successive days, Devine (52) observed no alteration of the blood sugar level, but nausea associated with definite nitrogen retention occurred. No pathological examination of the kidneys in these patients was possible. Among numerous clinical reports no instances of significant alterations in the blood glucose level have been observed after therapeutic doses in man.

The effect of stilbamidine in graded doses was also studied in rabbits by Devine (53). A single intravenous injection of half of the lethal dose (15 mg./kg.) did not cause any significant alteration in the blood glucose level, whereas the blood urea rose from 35 mg. % to over 100 mg. %. Single injections of 25 mg./kg. intravenously resulted in immediate collapse followed by prompt recovery in an hour. Within two hours of such an injection, a transient rise of almost 100% occurred in the blood sugar followed by a rapid fall to normal. No hypoglycaemia was noted to follow this glycaemia. However, the animals continued to reveal evidence of prolonged impairment of renal function, with either transient or fatal uremia. Death ensued in some animals after 8 to 10 days. Of some clinical interest are Devine's observations on the effect of repeated small intravenous doses comparable to the therapeutic doses used in man. Six daily injections of one-sixth the lethal dose (5 mg./kg.) produced no alterations in the blood chemistry. These observations would seem to indicate that in therapeutic doses, stilbamidine would not be expected to produce any clinical evidence

of hepatic or renal damage, unless such damage antedated the institution of therapy.

Essentially similar findings were obtained by Wien, Freeman and Scotcher (189), who performed extensive studies on the glycaemic effects of the four principle aromatic diamidines. The main action of subcutaneously administered stilbamidine and pentamidine was hyperglycaemic only when doses in the sublethal range were employed. Propamidine produced marked hypoglycaemia in large subcutaneous doses. Intravenous doses of propamidine produced hypoglycaemia, or in larger doses a transient hyperglycaemia followed by a fatal terminal hypoglycaemia. Phenamidine produced a delayed hyperglycaemia only after large doses indicating its relative lack of toxicity, compared to the other diamidines. In several animals studied by Wien and his associates, adrenalin hyperglycaemia was observed to be partially inhibited by stilbamidine. Ergotoxine reduced the hyperglycaemia caused by sublethal doses of pentamidine. Adrenalectomized animals revealed an inability to withstand propamidine-induced hypoglycaemia, as well as a failure to mobilize liver glycogen after sublethal doses of stilbamidine. These findings were believed to indicate that the adrenals "played some part in the glycaemic action of both stilbamidine and propamidine, this by sympathetic stimulation or by direct action on glycogenolysis." In an attempt to clarify these findings somewhat Wien studied the liver glycogen content four hours after intravenous injections of propamidine in varying dosage. No depletion of liver glycogen could be demonstrated four hours after a single injection in a large number of rats, but repeated injections produced marked depletion of liver glycogen, which was however, associated with signs of toxemia, anorexia and starvation. These observations have been supported by pathological examination of the livers of rabbits, mice, guinea pigs and cows.

The acute and chronic toxicity of stilbamidine was reported by Seager and Castlenuovo (156, 157). Oral administration of 100 mg./kg. daily to mice for 120 days resulted in fatty metamorphosis of the liver and in some instances marked degeneration of the renal convoluted tubules. When 10 mg./kg. was fed for one week distension of the central and interlobular veins of the liver as well as cloudy swelling and degenerative changes in the renal tubules was noted. Rabbits could tolerate 100 mg./kg. subcutaneously for only a few days while 10 mg./kg. daily killed all rabbits before 10 doses were administered. The most marked tissue alteration noted were fatty metamorphosis in the liver and extensive tubular degeneration in the kidneys. Some degeneration of the glomerular epithelium was also evident.

Impairment of liver function by the diamidines has been inferred by Dawes (51). He noted that adrenalin when injected into the portal venous system in animals treated with the diamidines caused a greater and more sustained rise in blood pressure than in a control animal. The response when adrenalin is injected into the jugular vein is lower in diamidine treated animals. The pressor effect observed when adrenalin is injected into the portal circulation of normal animals is usually lower than when it is injected into the systemic venous

system. This is attributed to inactivation of adrenalin by the amine oxidase in the liver. The action of the amidines as antagonists of this differential adrenalin response seemed to indicate some inactivation of the mono-amine oxidase in the liver. The amidines are not very strong inhibitors of the amine oxidase system (25) and Dawes therefore attributed the amidine action on adrenalin to a direct toxic action on the liver cells. Inhibition of the differential adrenalin effect may serve as a sensitive indicator of the hepatotoxic properties of chemical compounds.

A diffuse fatty infiltration of the liver has also been noted following maximum tolerated doses of either propamidine or pentamidine. The experimental observations in animals, that repeated small doses equivalent to therapeutic doses in man produce no discernible injury (53, 186), is supported by the fact that no published reports have yet appeared of liver damage occurring among several thousand patients thus far treated with one or several courses of the diamidines. A possible exception were several patients treated with old solutions of stilbamidine deteriorated by exposure to sunlight (111).

An evaluation of the experimental renal damage produced by the diamidines is hindered somewhat by the wide fluctuations in blood urea levels in animals suffering from the anorexia, syncope, and general toxæmia immediately following even therapeutic doses of the diamidines injected intravenously. Pathological examination of the kidneys of animals treated with large doses (20 mg./kg. rabbit) of propamidine and stilbamidine have revealed cloudy swelling, desquamation, and fatty degeneration of the renal convoluted tubules, whereas repeated doses of 2 to 5 mg./kg., equivalent to therapeutic levels in man, produced no histological changes whatsoever. After either the subcutaneous, intramuscular or intravenous routes of administration similar transient impairment of renal function was observed after maximal tolerated doses. Some animals were seen to die suddenly from uremia ten days after a single injection of a large dose of diamidine. These findings have led Arai and Snapper (14) to caution against the use of stilbamidine in patients with tubular nephrosis from multiple myeloma even though there is no evidence of renal toxicity following prolonged treatment with stilbamidine in man.

b. Respiratory and Vascular Effects. All four of the diamidines have a marked depressor action on the blood pressure as measured in intact animals, or in isolated physiological preparations (186). The drop in blood pressure is attributed to vasodilation in the smaller arteries and arterioles. The dilatation is believed to be a direct action on the blood vessel and independent of any central or nervous mechanism (186). This depressor effect is reduced somewhat in the intact animal by atropine. The pressor action of adrenalin is counteracted markedly by the diamidines. Marked tachyphylaxis in the vasodepressor response is obtained after rapidly repeated doses of any of the diamidines. In isolated heart-lung preparations, Wien (186) noted that concentrations of 1:2000 stilbamidine produced an increase in the rate and force of the heart beat, and higher concentrations had a moderate depressing action. However, the small magnitude and inconstancy of these changes suggested that the vasodepressor action of the diami-

dines was not due to any direct effect on the heart itself. Despite the frequently marked transient vasodepression following intravenous injections of the diamidines in man no evidence of cardiac toxicity per se has been reported. Wien (186) has also noted an antagonistic effect of calcium, when administered parenterally, on the fall of arterial pressure in animals. Oral feeding of calcium was incapable of protecting mice from the depressor action of stilbamidine. Atropine (14), and adrenalin, (141, 167), have been used to overcome the immediate syncopeal reactions in man. Neither of these agents, singly or in combination yielded satisfactory results. Repeated intramuscular injections, or a controlled continuous intravenous drip of the diamidino solutions have been tried to prevent the syncope often observed following single intravenous injections (104).

The effects of diamidines on the respiratory system are less striking than those on the vascular system, both as to degree and site of action. In rabbits and guinea pigs, large intravenous doses first produced a small increase, and later a decrease in respiratory rate. The slower type of respiration is also characterized by respiratory difficulty, deep breathing, simulating dyspnea. This has been attributed to the frequently associated drop in blood pressure. In rats and guinea pigs, Wien has concluded that there is no evidence of any direct depression of the respiratory center (186).

c. Central Nervous System Effects. In several short-term experiments Wien (186) reported little or no effect of stilbamidine on the central nervous system of guinea pigs, rabbits, or mice. No convulsions, depression of the respiratory center, or significant effect on the isolated knee-jerk preparation was noted. In these species the principle sites for the toxic action of sublethal doses appeared to be the liver and kidneys. However, in the dog, whose central nervous system is known to be very susceptible to the toxic manifestations of numerous pharmacologic agents, several observers have reported evidence of marked central nervous system damage from propamidine and stilbamidine. Kirk and Sati (115) observed transient paresis in dogs after large doses of propamidine. Lourie and Yorke (127), and Oastler and Fidler (143), noted that maximum tolerated doses of even freshly prepared solutions of stilbamidine produced vomiting, defecation, tremors, followed in some cases by foot drop, spastic paresis, decerebrate rigidity, mania, and later coma. Because of the frequency of late toxic neuropathy of the face in man (v.i.) the latter investigators (143) studied the chronic central nervous system toxicity of fresh stilbamidine solutions at therapeutic dosage levels. Ten dogs received 0.8-2.0 mg./kg. intravenously each day for four to twenty-one injections. Several dogs died and the remainder were sacrificed at the end of the period. Five dogs showed clinical signs of central nervous system damage verified post-mortem. The degree of cerebral damage could not be correlated with the amount of drug administered. The autopsied animals showed cerebral softening, inflammation of the meninges, together with hemorrhage and thrombosis within the cerebrum. Microscopically, cellular infiltration, thickening of the walls of blood vessels, and myelin degeneration was noted. The authors concluded that the cerebral lesions were a result of vascular spasm or anoxia, related partially to the rate of injection.

d. Other Pharmacologic Effects. A direct stimulating effect on the tone of the smooth muscle of the rabbit uterus and intestine has been described by Wien (186). Concentrations of the diamidines as low as 1/10,000–1/25,000 could elicit smooth muscle contraction. Guinea pig smooth muscle did not respond however. Inhibition of adrenalin stimulation of tissues after they had been exposed to dilute solutions of stilbamidine was also noted. Solutions of stilbamidine greater than 1:5000 produced spontaneous contraction of striated muscle. Wien (186) postulated that the drug increased the sensitivity of nerve muscle preparations by reducing the concentration of calcium and lowering the calcium-phosphorous ratio. In both dogs and rabbits Wien, Freeman and Scotcher (189) reported a slight fall in serum calcium and serum potassium within the first four or five hours after injection of stilbamidine, propamidine or phenamidine. In rabbits the reduction in potassium ion concentration exceeded the drop in calcium ion while in dogs both fell proportionately. They believed that these changes in calcium and potassium serum levels may account for the general stimulative effect of the diamidines on muscle. The generalized vasodilatation which could be modified by injection of calcium salts was not associated with the diamidine reduction of calcium and potassium concentrations.

e. Blood and Reticulo-Endothelial System. Even in massive fatal doses of stilbamidine or propamidine there was no evidence of any toxic action on the hematopoietic tissues of guinea pigs. Depression of the hematopoietic system has likewise not been reported in man. As measured by the Congo Red index (a procedure which is open to question), stilbamidine (90) did not alter the efficiency of the reticulo-endothelial system.

IV. THERAPEUTIC APPLICATION

1. Trypanosomiasis

a. Laboratory Animals. The early investigations of Lourie and Yorke (127, 192) established the aromatic diamidines as potentially potent chemotherapeutic agents in infections with African species of trypanosomes (*T. rhodesiense*, *gambiense*, *equiperdum*, *evansi*, *equinum*, *brucei*). *In vitro*, dilutions as high as one part in 200 million were trypanocidal.

In vivo against arsenic-fast and germanin-fast strains of *T. rhodesiense*, single doses of .005 mg./20 gm. mouse cleared the peripheral blood. Permanent cures were obtained with single injections of .01–.05 mg. Since most animals could tolerate 1.0 mg./20 gm. mouse intraperitoneally a minimal therapeutic index of 30 was often attained. This compared favorably with pre-existing drugs used in human trypanosomiasis (see Table 3) (184). In rats, rabbits, and guinea pigs infected with *T. rhodesiense*, the therapeutic index of the diamidines was similar to that found in mice. However, in mice infected with *T. congolense*, only the maximal tolerated doses of stilbamidine were effective (127) and frequently produced only temporary sterilization of the blood stream. Temporary sterilization or cures of infections with *T. lewisii* (8) have been obtained only by maximal tolerated doses of stilbamidine in rats. According to Lourie and Yorke (127) *T.*

crui, in either the blood-stream or tissue forms, was unaffected by maximal doses. It may be of considerable clinical importance that stilbamidine consistently displayed somewhat higher therapeutic indices (78, 81, 127) than the other diamidines against the more sensitive trypanosome species. Against the more resistant *T. congolense*, Fulton and Yorke (81) have reported that dimethyl stilbamidine (SN 311) (191), was twice as active as stilbamidine itself (187).

Drug resistance has been easily produced in both Trypanosome (192) and Babesia (37) strains within a period of several weeks by repeated subcurative doses. It extends across to all of the diamidine compounds. Even in the absence of drug and despite serial animal passage, such resistance has been maintained for several years.

The vast species difference among trypanosomes in response to diamidines has not been explained, although it is well known that the metabolism of *T. crui* and

TABLE 3

Comparison of Diamidines with Other Pharmacologic Agents in Trypanosomiasis

	MICE (<i>T. RHODESIENSE</i>)*			RABBITS (<i>T. RHODESIENSE</i>)**		
	M.C.D.	M.T.D.	T.I.	M.C.D.	M.T.D.	T.I.
Atoxyl.....	2.0	10.0	5.0			
Tryparsamide....	20.0	40.0	2.0	1.0	1.0	1.0
Stilbamidine.....	0.025-0.05	1.0	30.0	5.0	20.0	4.0
Propamidino.....	0.05-0.1	1.0	15.0			
Pentamidine.....	0.05-0.1	1.0	15.0	6.0	20.0	3.4

* mg./20 gm. mouse.

** mg./kg.

M.C.D. = Minimal Curative Dose.

M.T.D. = Maximum Tolerated Dose.

T.I. = Therapeutic Index = $\frac{\text{Maximum Tolerated Dose}}{\text{Minimal Curative Dose}}$.

T. lewisii differs markedly from that of the African species. For example, von Brand, Johnson, and Rees (30) have shown that the response to certain enzyme inhibitors varies widely. Cyanide inhibited the respiration of *T. crui* (30) and *T. lewisii* but according to several workers did not inhibit *T. rhodesiense* or *T. equiperdum* (41, 183). In addition, it has been known that the "African" species of trypanosomes utilize large amounts of glucose (40, 83) and oxygen whereas the former species utilize relatively little glucose (40). Whether these species differ in enzyme constitution has not yet been established, nor has any correlation been made between the known action of the diamidines upon certain enzyme systems and their therapeutic efficacy (see section II). Our understanding of drug action in man is still obscure. It would seem that worthwhile clues might be obtained by a study of those differences in Trypanosome metabolism which would account for the varied species response to diamidines.

b. *Stock Animals*. Synthalin administered at the maximum tolerated dosage has been shown to be ineffectual for horses and cattle infected with *T. congolense*.

Yorke (194) has stated that the therapeutic index is too narrow to offer any hope of eradication with stilbamidine. Daubney and Hudson (49) abandoned pentamidine after several animals relapsed on maximum doses and developed delayed liver atrophy. Among several recently studied aromatic diamidines, 4,4' diaminodino $\alpha:\beta$ dimethyl stilbene has been shown to be twice as active as any previous diamidine in *T. congolense* infections in mice (81, 187). After incomplete clinical trials, Carmichael and Bell (36) reported that in cows a few cures could be obtained with repeated maximum tolerated doses of 10 to 12.5 mg./kg. intravenously. For smaller laboratory animals this dosage produced immediate fatal reactions. Repeated smaller doses subcutaneously or intramuscularly were better tolerated and resulted in cure of a small proportion of these laboratory animals. More satisfactory results may perhaps be obtained by further study of the optimum dosage and route of administration. In general, the outlook for control of equine trypanosomiasis with the aromatic diamidines now in clinical use is poor.

c. *Man*. In the treatment of both Gambian and Rhodesian sleeping sickness, pentamidine, propamidine, and to a lesser extent stilbamidine, have been effective provided treatment was administered in the early stages of the disease (123). In both man and animals, the blood and the lymph-node puncture juice became sterile in from 48 to 72 hours from the onset of treatment (29, 93). It is doubtful whether the diamidines penetrate readily into the cerebro spinal fluid. Patients with marked spinal fluid changes (cell count above 30 wbc/mm.³) frequently showed little clinical improvement despite large doses of drug intravenously (29, 93, 134). It is probable the lack of effect in late cases was a result of irreversible pathological changes in the nervous system, plus poor penetration of the drug into the spinal fluid. The neurotoxic properties of large doses of diamidines may also be a factor in the poor results observed with cases in which therapy was delayed. The intrathecal administration of diamidines does not seem feasible because of their irritating action after local administration.

In patients classified as "early" (below 30 wbc/mm.³ in spinal fluid), cure rates of almost 100% have been reported (95, 123), whereas tryparsamide and antrypol have resulted in an 85-95% cure rate in this stage. With more advanced cases of the "intermediate" type (30-80 cells/mm.³ of spinal fluid), a 50-90% cure rate has been observed with stilbamidine (84, 122). When the spinal fluid cell count is above 80/mm.³ 50-80% cures have been reported with a pentavalent arsenical as compared to approximately a 30% cure rate produced by the diamidines (95). It is the consensus of most observers that, in the typical undernourished African native, pentamidine (94) and propamidine are distinctly less toxic (151) and more effective (122) than stilbamidine. They may be given intramuscularly with minimal local or systemic toxicity. The details of the reported clinical trials are presented in Table 4. It will be noted that drug response varies according to the virulence of the particular local strain, the nutritional status of the native populations, and the dosage schedule employed. Comparative therapeutic evaluation is not reliable unless the diamidines and arsenicals are tested with the particular local strain under consideration.

TABLE 4
Summary of Aromatic Diamidine Therapy in Human Trypanosomiasis

AUTHOR	SPECIES ¹ AND LOCATION ²	DRUG	DOSAGE** SCHEDULE	TOTAL DRUG EMPLOYED	ROUTE OF ADMINISTRATION	RESULTS			
						"Early"		"Late"	
						Treated	Cured	Treated	Cured
Bowlesman (29)	<i>T. Gambiense</i> (Gambia)	Stilbamidine	1 mg./kg. 2 × weekly	7-10 mg./kg.	I.V. or I.M.	20	17	11	3
Gilbert (34)	<i>T. Gambiense</i> (Northern Rhodesia)	Pentamidine	1.6-5.1 mg./kg.	8 daily doses	I.V. or I.M.			14	8
Harding (93)	<i>T. Gambiense</i> (Northern Nigeria)	Stilbamidine	?	3.2-6.3 mg./kg.	I.V.	3	3	10	1
Lourie (123)	<i>T. Gambiense</i> (Sierra Leone)	Pentamidine	0.8-1.7 mg./kg. or 50-100 mg./daily	4.8-13.8 mg./kg. 0.4-1.2 Gm.	I.V.	120	119	41	18
		Propamidine	50 or 75 mg./daily	0.4-0.6 Gm.	I.V.	24	22	15	7
		Stilbamidine	0.7-1.3 mg./kg. or 50-75 mg./daily	0.4-0.8 Gm.	I.V.	30	23	6	1
						COMBINED "EARLY" AND "LATE"			
Lawson (122)	<i>T. Gambiense</i> (Uganda)	Pentamidine	50-500 mg./daily	Ave = 1.0 Gm.	I.V.	53	41		
McLetchie (134)	<i>T. Gambiense</i> (Northern Nigeria)	Stilbamidine	0.7-2.2 mg./kg.	8.8 mg./kg.	I.V. or I.M.	8	7	6	2
Saunders (151)	<i>T. Gambiense</i> (Gold Coast)	Pentamidine	0.5 mg.-2 mg./kg.	0.8-3.0 Gm.	I.V. or I.M.	8	8	16	2
Van Hoof (181)	<i>T. Gambiense</i> (Belgian Congo)	Pentamidine	1-3 mg./kg.	0.3-0.95 Gm.	I.V. or I.M.	6	4	14	2
						COMBINED "EARLY" AND "LATE"			
Harding (94, 95)	<i>T. Gambiense</i> (Sierra Leone)	Pentamidine	50-100 mg./daily	0.4-0.6 Gm.	I.V.	193	141		
		Propamidine	50-100 mg./daily	0.4-0.6 Gm.	I.V.	55	40		

* "Early" cases are those with minimal spinal fluid changes, usually less than 30-80 cells mm³.

** Doses are expressed as hydrochloride salt of the diamidine.

¹ The species is presumably *T. Gambiense* but were not identified in these reports except as "Gambian," "Nigerian," etc.

Although all observers are agreed that the diamidines are valuable adjuncts in the control of human trypanosomiasis, there is no unanimity as to the drug of choice. Van Hoof et al. (181) stated that, if the first dose of tryparsamide did not clear the blood, pentamidine should be administered. Several thousand patients have been treated with pentamidine, propamidine or tryparsamide, alone or combined with suramin. Lourie (123) concluded that pentamidine and propamidine were the drugs of choice even against severe early forms of Gambian sleeping sickness. From an administrative aspect, diamidine therapy offers certain advantages over tryparsamide therapy. It may be administered intramuscularly by non-specialized personnel. It is economically more feasible. It may be given to children. These are important factors in the treatment of large communities of African natives. An inherent disadvantage is that a spinal puncture must be performed on all patients prior to the institution of therapy. If the spinal fluid protein is greater than 500 mg. per 100 ml. and if more than 80-100 cells/mm.³ are present, tryparsamide and antrypol offer better hopes for cure.

The diamidines compare favorably with the pentavalent arsenicals with respect to their clinical toxicity. During the treatment of approximately 500 cases, the only fatality attributed to the diamidines (pentamidine) was that reported by McComas and Martin (131). Their patient, with severe central nervous system involvement prior to treatment, developed status epilepticus and coma following a third intravenous injection of 100 mg. of pentamidine. It was difficult to determine whether a Herxheimer reaction, an inherent drug toxicity or pre-existing cerebral lesions were at fault. Autopsy revealed chronic perivascular cuffing in the cerebrum which was believed to have antedated diamidine therapy. Pentamidine has been used in patients who had previously shown signs of arsenic poisoning without any untoward effects.

At present careful studies of dosage schedules, routes of administration and duration of treatment are being conducted. In general, most investigators have given 8 to 14 injections of pentamidine in doses of 1 mg./kg. I.V. and 2 mg./kg. I.M. Stilbamidine, because of its greater toxicity, has been supplanted by propamidine or pentamidine in the treatment of trypanosomiasis. No reports of therapeutic results with the newer methylstilbene derivatives have been noted.

d. Prophylaxis. In guinea pigs, Van Hoof et al. (181) observed that *T. gambiense* may be cleared from the peripheral blood and gland juice with a single dose of pentamidine as low as 2 mg./kg. With this dose, the blood-free interval varied from 11 to 125 days before relapse ensued. This suggested to Van Hoof the possibility of prophylactic action in man. Two volunteer laboratory workers were given a single dose each of pentamidine, 2 mg./kg. and 3 mg./kg. respectively. For the following months, they were bitten every two or three days by heavily infected Tsetse flies. After periods of nine and twelve months the volunteers contracted an infection. However, the clinical course was atypical. The blood cultures and smears were frequently negative and the temperature remained normal. Both volunteers were cured with suramin. In a similarly treated group of natives from a densely infected area of the Belgian Congo, Van Hoof, et al.

(182) noted no infections during a three month period, whereas 2.5% of a control group developed new infections during the same interval. Fulton stated that this type of protection was a manifestation of inapparent immunization also, rather than one of true prophylactic action. Mice so treated resisted massive inoculations of *T. rhodesiense* and *T. congolense* for only two to four weeks. When inoculations of light suspensions of trypanosomes were used, protection for periods of 8 to 24 weeks was observed. Among mice not completely protected, the infection often took a prolonged irregular course. Thus, prophylactic activity probably existed for a variable period after administration of the diamidines. According to Fulton this period was proportional to the therapeutic index of the drug and its rate of excretion. Fulton and Lourie (76) investigated the immunity of mice cured of trypanosome infections. The strains of trypanosomes employed were:

- a. *T. rhodesiense*
 - 1. laboratory strain
 - 2. atoxyl-fast strain
 - 3. suramin (antrypol, Bayer 205) fast strain
- b. *T. equinum*
- c. *T. equiperdum*
- d. *T. congolense*

The mice were treated with 4,4' diamidiano $\alpha:\beta$ dimethyl stilbene or reduced tryparsamide thioglycollate. Three types of response were noted after reinoculation. The first type was reinfection after the usual incubation period indicating no immunity. Slight immunity was evidenced by infection after a delayed incubation period while absence of infection indicated definite immunity. No cross immunity between the various strains of trypanosomes was observed. Homologous immunity was observed to varying degree. No immunity between *T. rhodesiense* and the suramin fast variant was observed although such immunity was complete versus the atoxyl fast strain.

2. Babesiasis

Lourie and Yorke (128) first demonstrated marked action of most of the diamidines against *Babesia canis* infections in puppies. These drugs appeared to be far superior to either Trypan blue, or acaprin,⁵ the two pre-existing therapeutic agents. However, if subcurative doses were given, a rapid drug resistance developed which persisted in some strains for many years despite frequent animal passage. Fulton and Yorke demonstrated drug resistance extending across to the several diamidines in use (77). Infections with tick fever (*B. canis*) in dogs have yielded very satisfactorily to treatment even among advanced cases in which hemoglobinuria and shock were present. Daubney and Hudson reported that in 16 dogs satisfactory cures were obtained with stilbamidine (50). Transient toxic reactions such as swelling of the face, hyperesthesias, dyspnea, and tremor were observed but these signs usually disappeared after a half hour. In 116 animals Carmichael and Fiennes (37) reported that propamidine (5 mg./kg.

⁵ Acaprin is 6:6'-(di-N-methyl quinolinium-methosulfate) urea.

I.M. once or 2.5 mg./kg. twice) resulted in 95% cures. The immediate transient toxic reactions were less marked than with pentamidine. Relapses were easily treated. Subsequently Carmichael (34) reported that in a series of 25 cases phenamidine (M & B 736), in doses of 10 mg./kg. subcutaneously, was tolerated better than any of the previous diamidines in use. Most animals showed clinical improvement, sterilization of the blood, and febrile lysis within 12 to 24 hours from onset of therapy. These observations were confirmed by Pierse (144). Similar favorable results occur in *B. ovis* infections in goats (7) and *B. bigeminus* infections in calves treated with stilbamidine, but in *B. cabelli* infections (biliary fever of horses) the therapeutic index appeared small in the few cases treated by Daubney and Hudson (50).

Adler and Tchernomoretz (7) observed no effect on experimental infections of *T. annulata* (Theileriosis), *A. ovis*, and *A. marginata* infections (Anaplasmosis) of calves and goats.

3. Leishmaniasis

a. Leishmaniasis in Animals. At the suggestion of Yorke, who first observed the trypanocidal and leishmanocidal activity of the diamidines *in vitro*, Adler and Tchernomoretz (8) studied the action of stilbamidine on a strain of *L. donovani* in Syrian hamsters. With repeated single doses of from 2.5 to 20 mg./kg., stilbamidine promptly cured both mild and severe infections in these animals. *In vitro*, stilbamidine inhibited growth at a concentration of 1:20,000 whereas neostibosan permitted growth in concentrations of 1:100. These same authors extended their observations (9) to hamsters infected with the organism of Mediterranean cutaneous leishmaniasis, *L. infantum*. Somewhat reduced therapeutic activity was noted than upon *L. donovani*, thus supporting clinical observations to this effect (v.i.). In a recent comparative study of the three principal diamidines, Adler, Tchernomoretz and Ber (10) could find no correlation of *in vitro* with *in vivo* tests. Collier and Lourie (46) found that the *in vitro* sensitivity of a leishmanial strain varied with the temperature employed for testing. At 24°C. the anti-leishmanial titer was one to 50,000 for phenamidine, 250,000 for pentamidine and 125,000 for stilbamidine. At 34°C. these titers were 500,000, four million, and two million respectively. These authors present an excellent discussion on the relationship of structure of chemical compounds to their anti-trypanosomal and anti-leishmanial activity. Although pentamidine and propamidine were equally as effective as stilbamidine *in vitro*, they were incapable of eliminating severe infections of *L. donovani* which would otherwise yield to stilbamidine. Phenamidine (4:4' diamidino-diphenyl ether) did not control mild infections in Syrian hamsters even after repeated injections of 10 mg./kg. (9). Among the newer aryl diamidines, Fulton (72) has noted that the monomethyl and dimethyl derivatives of stilbamidine were almost as effective as stilbamidine itself against *Leishmania donovani* in hamsters. (These methyl derivatives were more effective than stilbamidine against *T. congolense*.) In a few preliminary experiments a 2 hydroxy-stilbamidine derivative proved equal to stilbamidine itself.

So far no reports have been noted relative to the prophylactic action of the

diamidines in Leishmaniasis. The relationship of drug dosage to the establishment of diamidine-resistant strains of Leishmaniasis in animals has also not been reported.

b. *Human Leishmaniasis*. The anti-leishmanial action of the diamidines in animals was confirmed in patients suffering from Indian Kala-azar. After a short course of stilbamidine, consisting of 1 mg./kg. intravenously for eight days, Adams and Yorke (3, 4) and Wingfield (190) observed sterilization of blood and bone marrow and prompt remission in fever, anemia, and hepatosplenomegaly in three British seamen. Two of the patients suffered from severe infection previously not responding to pentavalent antimony. Following these accounts, Napier et al. (138, 141) treated a series of 101 patients including many antimony resistant cases. With a course of 8-12 intravenous injections of 1-2 mg./kg., there were 98 cures, two relapses and one death. Immediate syncopal reactions were troublesome with the single syringe technique but did not necessitate interruption of therapy. These results definitely established stilbamidine as a potent clinical agent for the therapy of Indian Kala-azar. However, against the more resistant Sudanese Kala-azar, the same dosage yielded only 24 cures among 28 patients as reported by Kirk and Sati (113, 114, 115). These latter authors noted permanent cures in two cases of espundia. In Mediterranean infantile leishmaniasis, Adlar and Rachmilewitz (6), and Süsskind and Roth (174) reported that stilbamidine was an effective agent when sufficiently large doses were administered over a period of several months (30 injections of 1-2.5 mg./kg.). In one boy of 5½ years, a relapse occurred after a total of 1.17 gm. stilbamidine had been given. A subsequent course of .54 gm. urea stibamine was ineffectual. However, a second course of 3.75 gm. of stilbamidine effected complete recovery. Another patient, a boy of 10 years with a serious disease of three years duration was treated for five months with daily intravenous injections of 50 mg. stilbamidine. A total dose of 5.5 gm. was administered without serious toxic reactions. Although these patients and others (57) were cured by stilbamidine, it was apparent that only prolonged treatment with this drug would eradicate the majority of Mediterranean strains of Leishmaniasis.

Prolonged administration of stilbamidine has been shown to be associated with the appearance of a late chronic toxic neuropathy of the trigeminal nerves. Because of the acute and chronic toxicity of stilbamidine and despite the superior therapeutic activity of stilbamidine in animal leishmaniasis, the clinical usefulness of other diamidines has been studied.

In a series of 32 cases, Napier and Sen Gupta (140) observed that pentamidine cured 19 of 31 ordinary cases. In contrast to stilbamidine, intravenous administration of pentamidine produced few untoward immediate or late reactions. In Sudanese Kala-azar, Kirk and Sati (115) observed 10 cures among a group of thirteen patients. Both oro-pharyngeal leishmaniasis (105) and extensive skin lesions (112) have been reported to yield promptly to pentamidine therapy.

In summarizing their experiences Napier and Sen Gupta (140) among others (1, 13, 85, 160, 164, 177, 178) believed that pentamidine was inferior to stilbamidine and to the best pentavalent antimony preparations. Stilbamidine was

deemed the treatment of choice in patients with intercurrent tuberculosis in whom antimony was contraindicated (161). Because of the neuropathic sequelae of stilbamidine, pentavalent antimony was preferred for untreated cases of Indian Kala-azar in whom a cure rate of approximately 95% had been noted over a period of many years.

The reported cases of propamidine-treated Kala-azar have thus far been limited to three in number. Adams (2) reported a cure in a single case of Indian Kala-azar. This case was treated with only nine intramuscular injections of 100 mg. each, whereas Kirk and Sati reported (114) one death and one permanent recovery, with a slightly larger dosage schedule.

More recently Sen Gupta (162, 163) studied the therapeutic action of phenamidine (M & B 736), (4:4' diamidinodiphenyl ether), in Indian Kala-azar. Treatment consisted of ten daily injections of 1.0 mg./lb. intravenously repeated after a ten day rest period. Among 21 adequately followed patients, there were 16 cures and five relapses. Sen Gupta concluded that phenamidine was non-toxic but inferior to pentavalent antimony and stilbamidine. No case of trigeminal neuropathy or peripheral neuritis occurred. One patient developed malarial fever during treatment.

While a final evaluation of diamidines in the treatment of leishmaniasis must await further study of the newer derivatives, experience thus far seems to indicate that the most potent leishmanocidal agent, stilbamidine, is too toxic for general use. Its use will probably be confined to antimony resistant cases of Indian Kala-azar, (177). In the treatment of Mediterranean, Chinese and Sudanese Kala-azar, stilbamidine may yield results so superior to antimony (13, 97, 110, 159) that, despite the incidence of late toxic neuropathy, it will be adopted as the drug of choice. Whether the production of diamidine resistant strains by the present dosage techniques will be an important factor in the future remains to be seen. The apparent development of resistance to stilbamidine after a course of twelve injections of 150 mg. intravenously has been observed in patients infected with Mediterranean Kala-azar (personal observation, E.M.G.). Since such resistance applied to the four diamidines tested, both *in vitro* and *in vivo*, the entire usefulness of the diamidines may be sharply limited unless satisfactory methods are developed for the prevention of drug-resistance by various therapeutic techniques.

4. Malaria

Christophers and Fulton (42) demonstrated that a considerable number of monkeys otherwise fatally infected with *P. knowlesi*, could be saved with N-undecane 1-11 diamidine. The drug was ineffective in *P. relictum* infections of canaries at maximum doses, and was somewhat less effective and slower acting than atabrin in *P. knowlesi* infections. However, it was deemed worthy of clinical trial in man. Glyn-Hughes, Lourie and Yorke (86) studied 18 patients therapeutically infected with simple tertian malaria, and one patient with quartan malaria. The patients were treated with doses of 25 mg. three times daily intravenously or 50 mg. twice daily orally. One or two paroxysms were usually

noted after treatment had been instituted. Parasites disappeared from the blood in from 3 to 6 days. Relapses occurred in from one to six months. Undecane diamidine was thus inferior to atebirin in both man and animals. It is of interest that oxygen uptake of *in vitro* suspensions of "parasite substance" (*P. knowlesi*) studied by means of the Barcroft manometer, were inhibited by undecane diamidine to a much greater extent than by either quinine or atebirin.

The aromatic diamidines (stilbamidine, pentamidine) were as ineffective as undecane diamidine in *P. relictum* infections in canaries. Pentamidine delayed infection for brief periods (69). In monkey malaria both stilbamidine and pentamidine had curative activity (69). Das Gupta and Siddons (48) have confirmed the general antimalarial effect of the diamidines. They treated 20 monkeys heavily infected with *P. knowlesi*. In the otherwise fatal primary attack, doses of 1 to 5 mg./kg. of stilbamidine saved 16 of the 20 animals. Relapses were somewhat more frequent than were usually observed after atebirin treatment. Although no experimental trials in naturally acquired human malaria have been reported, Sen Gupta (163) has observed that, in a highly endemic area, malaria is seldom seen in patients undergoing stilbamidine treatment for kala-azar. From this evidence, it has been inferred that the diamidines display some plasmodicidal activity at therapeutic levels in monkeys and man. It seems improbable that the diamidines will prove effective in *P. falciparum* infections. The activity of the aromatic diamidines versus various experimental malarial infections is summarized in a report by the Committee on Medical Research of the Office of Scientific Research and Development (191).⁶

5. Filariasis

The effect of stilbamidine on filariasis has been reported by Snapper and Merliss (168). Two cases of infection with *W. bancrofti* were completely uninfluenced by treatment with stilbamidine in doses of 150 mg. intravenously.

6. Schistosomiasis

At the suggestion of Yorke, Stephenson (173) treated nine cases of Schistosomiasis. Two were regarded as cured, two were improved and five were unaffected by a course of 15 injections of 150 mg. of stilbamidine diisethionate. Cawston (38) noted no effect whatsoever on several patients treated with the drug. The authors conclude that stilbamidine is probably of no value in the treatment of Schistosomiasis.

7. Rheumatoid Arthritis

With the premise that the diamidines might influence some unknown and presumably infectious agent in rheumatoid arthritis, Rosenberg (148) has treated

⁶ Stilbamidine—SN 251.

Phenamidine—SN 9,404.

Propamidine—SN 6.

Pentamidine—SN 9,406.

Data on other aromatic diamidines screened in the Malaria Survey are included (191).

four cases with a total of one gram of stilbamidine and two with a similar dosage of propamidine. No alterations were observed in either bone pain or clinical symptoms. This observation and those of Snapper's (166) confirm the fact that stilbamidine is not a general analgesic for the suppression of bone pain either by action on the central nervous system or on peripheral nerves.

8. *Osseous Metastatic Carcinoma*

Snapper (165) and Greenspan (personal observation) did not observe any effect on bone pain or the clinical course of the disease when several patients suffering from generalized bone metastasis from carcinoma of the breast were treated with stilbamidine.

9. *Multiple Myeloma*

Because both kala-azar and multiple myeloma are diseases characterized by marked alterations of blood proteins, and because he had observed the remarkable therapeutic action of stilbamidine on the former disease, Snapper (166) first instituted the administration of stilbamidine for the treatment of patients with multiple myeloma. From the outset it was soon apparent that the most outstanding clinical effect of the drug was the dramatic relief of the severe generalized bone pain so characteristic of this disease (12, 135, 104, 167). Remission in bone pain occurred usually between the fourth and sixth daily injection and remained more or less permanent. In a large number of patients, Snapper (167) and Snapper and Schneid (169, 170) witnessed the appearance of large basophilic inclusion granules in the cytoplasm of the myeloma cells. These were visualized with ordinary Wright or Giemsa stains of bone marrow spreads. The cytoplasmic granules were observed to appear between the third and sixth week after the onset of therapy, when amounts varying from 1.8 to 3.6 grams of stilbamidine had been administered.

A series of studies in which specific staining techniques, ultraviolet absorption spectra, ultraviolet microscopy with monochromatic light, as well as preparations of the specific enzyme, ribonuclease, were used, led Snapper and his associates (171) to infer that the precipitated granules were composed of complexes containing ribose nucleic acid and stilbamidine. It is noteworthy that among a series of more than 30 patients, granules appeared only in those patients manifesting hyperglobulinemia, Bence-Jones proteinuria or both (approximately 60% of the series) (170). Relief of bone pain was noted in almost all patients however.

Aside from the relief of bone pain, the clinical course of patients treated with stilbamidine did not reveal any clear-cut difference from the course usually observed among untreated myeloma patients. For example, in no case was there evidence that the myeloma cells were so damaged as to lead to cell death. There was no indication that myeloma cells were reduced in the bone marrow as the myeloma cells remained in their approximate former ratio to other cells. There was no apparent alteration of the blood proteins. Nevertheless, in no patients in this group did Snapper (167) observe progressive expansion of osteolytic bone lesions after the onset of stilbamidine therapy.

Snapper (167, 170) advocated a low-protein diet and believed that this was requisite for good results with stilbamidine therapy. Presumably the low protein diet avoids the antidiarrhoeal effect of proteins rich in nucleic acids. In two patients, pentamidine was stated to give relief of bone pain after stilbamidine had failed but in other cases the opposite was noted. After pentamidine administration, granules in myeloma cells were not observed.

These observations are difficult to interpret. The effect of stilbamidine on prognosis is also not yet clear. Cures are not claimed nor is there any objective evidence concerning the extent of clinical or pathological arrest of the disease. Mellinger (135) reported a case treated at the Massachusetts General Hospital with 50 mg. intravenously every other day for 10 days and noted a diminution in pain.

A follow-up averaging ten months in Snapper's series of cases revealed that four of fifteen patients had died (14, 166, 167), two of these with myeloma kidney, one with thrombopenia, and one with paraplegia. In a recent large series of myeloma cases not treated with stilbamidine, the average life expectancy was 18.8 months (17). Several years or more will be necessary before proper evaluation can be made of Snapper's results with stilbamidine. Nevertheless, the important observation has been made that this new therapeutic agent has the ability to influence specifically the morphology, and presumably the metabolism, of a specific human neoplastic cell type. There is some evidence that other aromatic bases may have similar potentialities with respect to tumor metabolism and that the formation of drug-precipitates within certain cells of the hematopoietic tissue is not unique with stilbamidine. For example, basophilic inclusion bodies have been reported to occur in the cytoplasm of lymphocytes of nine different species of mammals and avians following the administration of acridine, an acridine dye with fluorescent properties similar to stilbamidine. It is noteworthy that the N-acridine group, together with the quinolines, phenazines, diamidines, and quaternary ammonium derivatives, form a large group of aromatic bases which are being carefully studied as possible chemotherapeutic agents of animal tumors. Dickens (55) surveyed the metabolic effects of such compounds. Using respiratory studies on normal and neoplastic tissue cultures immersed in solutions of a given drug, he noted that, among a large number of compounds, N-undecane 1-11 diamidine inhibited the Pasteur effect more completely than "any compound yet known." N-undecane 1-11 diamidine had already been known as the alkyl analogue of stilbamidine with pharmacological properties similar to the latter compound. Later, as previously mentioned in this review, Haddow, Harris and Kon (91, 92) observed inhibition of transplanted Walker carcinoma, and other transplanted tumors in rats treated with 4:4' dimethylaminostilbene. More recently Kopac (118, 119, 120), has observed arrest of mitosis and selective destruction of tumor cells in tissue cultures, as well as denaturation of specific nucleoproteins by physiological concentrations of stilbamidine. With this pharmacologic and experimental evidence it seems entirely possible that Snapper's clinical results in multiple myeloma may represent a rational extension of the observed action of stilbamidine as a tumor inhibiting agent with perhaps more than theoretical implications.

Snapper (personal communication, July, 1948) has treated six cases of multiple

myeloma with 2-hydroxystilbamidine. Basophilic granules have appeared in the cytoplasm of the myeloma cells. These granules contain ribose nucleic acid but combination with 2-hydroxystilbamidine, as with stilbamidine, has not yet been demonstrated. Therapy with this stilbamidine derivative has been well tolerated even when higher dosage than that employed with stilbamidine has been used. Trigeminal anaesthesia has not been noted with 2-hydroxystilbamidine although the period of observation since therapy was completed is relatively short.

A summary of 194 cases of multiple myeloma, excluding 26 cases reported by Snapper (166, 167), treated with the di-isethionate salt of stilbamidine has been prepared by the Medical Division Merck & Co., Inc. (R. C. Pogge, April 1948). The patients were treated by 150 different investigators and it is not feasible to abstract this survey in detail. Approximately 63.0 per cent of the patients, in whom pain was a predominant symptom, experienced some relief. Complete amelioration of pain for a significant period was reported in 24.7 per cent of these patients. Serial X-Ray examinations of 99 cases revealed no change in the size or character of the lesions in 76 while progression of lesions was demonstrated in 28 patients. There were 5 instances in which roentgen examination indicated a decrease in the size of the affected area. Results of sternal marrow puncture were essentially the same as those noted by Snapper. Basophilic inclusion bodies following treatment were observed but there was no evidence that the number of myeloma cells decreased. Prolongation of life cannot be determined from the data presented. The chief reactions encountered among 209 patients treated with stilbamidine di-isethionate administered either intravenously or intramuscularly are tabulated in this report. Reactions of such severity as to contraindicate further treatment with stilbamidine were encountered on only three occasions.

10. Antibacterial Activity

Following Fuller's (67) demonstration of the bacteriostatic and antibacterial properties of propamidine against the Gram-positive cocci, an extensive clinical trial in the treatment of war wounds was made in Britain during 1942-43. Thrower and Valentine (175) first reported that treatment of purulent open war wounds with propamidine compared favorably with sulfathiazole when *Staphylococcus aureus* and *Streptococcus hemolyticus* were the causative organisms. Moreover, propamidine was not inhibited by the purulent exudate. A one-tenth per cent solution of propamidine did not inhibit phagocytosis, which, however, was slightly reduced by 0.2% solutions and completely inhibited by 0.4%. *In vitro* this inhibition was reversible by washing leucocytes even after three hours incubation with the drug. No hemolysis of red blood cells occurred at these higher concentrations.

A series of 50 patients were satisfactorily treated prior to plastic surgery with the sole application of a water soluble methyl cellulose gel containing 0.1% propamidine applied over a ten day period to infected war wounds (175). Although these results were deemed favorable, Elson (61) has pointed out that the relative acidity of the cellulose gel is far from the optimum pH at which the

diamidines exert their maximum antibacterial action. Nevertheless, sterile wounds were produced in almost all cases, except in infections with *Ps. aeruginosa* or with *B. proteus*. In a few cases in which higher concentrations or prolonged applications were necessary, there was a tendency towards irritation and some necrosis at the edges of granulation tissue. Excellent healing occurred in these cases after withdrawal of the drug. Similar observations were extended by McIndoe and Tilley (132) who noted rapid improvement within forty-eight hours in Streptococcus infections and somewhat slower action against Staphylococcus infections. Propamidine in a Mumford Base (lanette wax and paraffin or white oil) was especially suitable for application to infected wounds prior to plastic surgery. Butler (33) confirmed these observations and successfully treated several cases of Staphylococcal arthritis by injection of a 1.0% propamidine-saline solution into the infected joint space. Gairdner (82) treated an infant with staphylococcal pyopneumothorax with local irrigations of propamidine together with parenteral sulfapyridine. That this method of treatment is not without danger is shown by an experimental study by Frankel, Lee and Houlihan (66) on purulent staphylococcal arthritis in rabbits. Comparable series of rabbits were treated by local intracapsular injections with penicillin and with propamidine. Both agents produced sterile joints, but propamidine permitted less destruction and exudation into the joints. However, all propamidine treated animals died in from three to six days after cessation of treatment presumably as a result of systemic absorption of toxic amounts of the drug. The latter was administered in doses of 0.75 cc. of a 1.0% solution for five successive days.

In experimental Clostridial infections of mice, McIntosh and Selbie (133) noted that propamidine compared favorably with the sulfonamide drugs and in some instances was as effective as penicillin. In the treatment of several dozen severely burned air-raid casualties, Morley and Bentley (136) have agreed that propamidine gel or propamidine in a Mumford base afforded excellent polyvalent bactericidal properties against the usual Gram-negative organisms. Rapid separation of slough occurred together with no impairment of epithelization. Kohn, Hall and Cross (116) stated that Thiersch and pedicle grafting was possible "much earlier than with any other methods used before." However, according to Clark (43), propamidine yielded only 62% sterile burn wounds compared to 76% for penicillin. An experimental study of toxic effects in animals treated for burns supports the clinical impression of the comparative innocuousness of topical application of propamidine. In various animals, Allen, Burgess and Cameron (11) could not find any significant signs of absorption from large burned areas (one-eighth total body surface) or from normal skin. In a small series of tropical ulcers treated in the Solomon Islands, Forrest (64) reported complete healing with propamidine. In several patients external otitis responded to 0.1% propamidine in Mumford Base. According to Robson and Scott (147) experimental streptococcal infection of the rabbit cornea has been easily controlled with propamidine. Some irritation of the cornea was noted after prolonged application of concentrations of 0.6% propamidine. In another instance, Valentine and Edwards (180) reported that a 0.15% propamidine diisethionate solution was

effective in curing a human epidemic of angular conjunctivitis, after conventional agents had failed. Lastly, Cawston (39), a physician himself, after self treatment, suggests the use of insufflated propamidine powder into infected sinus cavities. From this evidence it may be assumed that propamidine, and possibly the other diamidines, are surface antiseptics whose principle limitation is a tendency towards irritation of normal tissues after prolonged application. No published data have appeared with respect to the efficacy of the diamidines in systemic bacterial infections. Because of their known toxicity the diamidines should be reserved at present for experimental chemotherapy of infections resistant to the conventional antibiotics and other agents. Until further clinical studies have been performed upon surface absorption, tolerance and excretion in man, the exact clinical indications for the use of the diamidines will remain unresolved, chiefly because of the subordinate clinical interest in synthetic antiseptics since the advent of the antibiotics, penicillin, streptomycin, gramicidin and polymyxin.

11. Fungistatic Properties

The general anti-microbial spectrum of propamidine has been studied *in vitro* not only against bacteria, but also against a large number of pathogenic fungi. By means of the agar streak plate technique, in which a solidified yeast extract agar containing varying amounts of the drug was inoculated with suspensions of fungi, Elson (61) observed that certain pathogenic fungi were inhibited by low concentrations of the drug. A two to four fold increase in activity was noted in media at pH 6.8 compared with media at pH of 5.0. In general most of the pathogenic fungi were inhibited by concentrations of 1 to 5 mg/20 cc. of propamidine in agar. However, *Achorion schoenleinii*, *B. dermatidis*, *Sporotrichum schenckii*, and *T. sulfureum* were inhibited by concentrations as low as 0.018 mg/20 cc. agar. This compares favorably with the bacteriostatic activity of propamidine on Staphylococci and Streptococci. Stilbamidine when tested *in vitro* against *Histoplasma capsulatum* was effective in a concentration of 10 mg. per 100 milliliters of medium. Neoarsphenamine did not show comparable inhibition until 10 times this concentration was attained, while penicillin, streptomycin, neostam (stibamine glucoside) had no effect (155).

The incorporation of soya lecithin, a phospholipid, into the medium inhibited the fungistatic activity of propamidine (60, 61). This was also noted in studies on the bacteriostatic properties of this diamidine. The high concentration of lecithin necessary to inhibit the relatively small amount of propamidine would suggest some impurity such as lipositol in the lecithin fraction as the active inhibitory agent (146).

No clinical trials of diamidines in the treatment of systemic or cutaneous fungous disease has been reported. Experimental therapy of the latter would seem warranted.

12. Spirochaetal Disease

Lourie and Yorke (127) noted no therapeutic action whatsoever in mice infected with *Spirochaeta recurrentis*, and *Spirillum minus*. This was supported

by Lourie's report (124) that in human cases of yaws treated in Sierra Leone, stilbamidine was ineffectual.

13. *Virus and Rickettsial Disease*

No study of experimental diamidine therapy of virus or rickettsial disease has appeared in the literature.

14. *Clinical Toxicity*

Several thousand patients, including many poor-risk, chronically ill Asiatic and African natives, have already been treated with one or more of the diamidines. No instance of a fatality has been attributed to properly prepared solutions of these drugs. In the early stages of World War II, however, Kirk (111) and Bowesman (29) reported more than a dozen cases of fatal delayed poisoning occurring 1-3 months after treatment with stilbamidine (3-5 gms.). In five of ten cases studied by Bowesman (29), and in several patients studied by Kirk (111), autopsy examination of the liver revealed central necrosis, fatty degeneration, cellular pyknosis, as well as other degenerative cellular changes. No evidence of massive hepatic necrosis of the acute yellow atrophy type was noted. Renal damage, as evident by moderate to severe degenerative changes in the tubules, was present in all these patients, whose death was attributed to combined hepatic and renal injury. The solutions of stilbamidine used in these cases was manufactured in England and shipped by air to Africa. Kirk (111) and others (16, 70, 79, 100) have shown that such solutions may contain toxic deterioration products produced by ultraviolet light. Since this accident, stilbamidine has been supplied as a powder in which no such deterioration has been observed to occur months or years after storage in the dark. Freshly prepared solutions of stilbamidine have given no evidence of clinical hepatic or renal injury. However, both immediate and late toxic reactions of a less serious nature have been observed to follow the administration of the diamidines. These have been sufficiently troublesome to influence the choice of drug in the treatment of Kala-azar and Trypanosomiasis (177, 194).

a. Immediate Reactions. During or immediately following the intravenous injection of stilbamidine, pentamidine, or propamidine solutions, many or all of the following symptoms and reactions have been elicited or observed (in approximate order of decreasing incidence): Fall in blood pressure, rapid thin pulse, facial flush, dizziness, salivation, sweating, headache, nausea, vomiting, dyspnea, formication, syncope, lethargy, fecal and urinary incontinence, epileptiform twitchings (pentamidine), and edema of the eyelids and face (1, 123, 140, 141, 160). Kirk (111) reported fewer immediate reactions after pentamidine than after stilbamidine but others did not confirm this observation (123). These reactions were usually transitory and disappeared in from 10-30 minutes. They were uninfluenced by atropine (141), adrenalin (141, 167) or benadryl (167). They were much less severe but not completely eliminated by recourse to intramuscular injection. Lourie (123) noted that severe immediate reactions may be partially eliminated by giving the injections slowly. By means of a slow intravenous drip

completed in a 20 minute period, Haedicke and Greenspan gave doses of 150-300 mg. (2.5 to 5 mg/kg.) of stilbamidine intravenously in 250 cc. volume daily for several weeks without any immediate reactions (104). With the single syringe technique, reactions occurred in about 70% of the patients (122). For clinic patients intramuscular injections would be more feasible and therefore preferable.

In several patients with Kala-azar, a modified Herxheimer reaction (141) occurred after injections of the diamidines. Within six hours after the first injection a marked febrile response and general toxic state was observed in patients who were severely ill with other systemic diseases. No such reaction has been observed among multiple myeloma patients treated with stilbamidine (165).

b. Late Reactions. The late chronic toxicity of the diamidines has been exclusively confined to a unique neuropathy which is so unlike any other drug-induced or naturally occurring syndrome, except possibly that following trichloroethylene or streptomycin, that Napier and Sen Gupta (139) suggested it be designated specifically as "diamidinostilbene neuropathy." Two to five months after a course of stilbamidine, patients gradually observed the appearance of progressive sensory changes consisting of paraesthesia, anesthesia, hypalgesia, and numbness confined usually to the face area (44, 45, 139, 159). Examination invariably revealed the presence of a dissociated anesthesia in which there was decreased sensibility to light touch, but pain, temperature, and pressure sense were intact. The trigeminal distribution radiating from the tip of the nose in a centrifugal pattern was usually involved. On occasion the same findings have been reported to extend to the neck and waist. Some patients have shown slight loss of pain sensation. Often these symptoms slowly disappeared after a period of several months, but in others they seemed to persist indefinitely. In the largest series, Sen Gupta (159) reported 17 instances of neuropathy among 104 patients followed. It is likely that the actual incidence of neuropathy is higher when cases are followed carefully. Snapper (165) observed over 50% incidence of neuropathy whereas Collard and Hargreaves (45) reported almost 100% incidence of this complication among British servicemen. Among the latter a high proportion of cases showed neuropathy extending to the waist.

The mechanism of stilbamidine neuropathy is totally obscure. Dosage, route of administration, speed of injection, or severity of immediate reactions do not appear to influence the occurrence of this complication (159). Sen Gupta (159) postulated that a lesion was produced in the principal sensory nucleus of the trigeminal nerve. In support of this contention are the findings of Oastler and Fidler (143) that dogs showed marked pathological changes in the meninges, cerebrum and brain stem which appeared to be secondary to vascular spasm or anoxia. No localization whatsoever was observed in the pons. Snapper has cautioned against the exposure of patients to excessive ultra-violet light on the premise that some stilbamidine remaining in the skin may be altered and the toxic products thus formed may initiate selective nerve injury.

c. Local Reactions. Solutions of diamidines may produce severe local reactions at the site of injections. Thrombophlebitis (141) has been observed frequently after administration of concentrated solutions by vein. In very high doses in

rats and other animals (20 mg/kg.) (158), slough and necrosis has been observed, but in the therapeutic doses in man, intramuscular injections usually produce only slight local inflammation and pain at the local site (123, 141). Stilbamidine salts, especially the hydrochloride, are less soluble and more irritant locally than pentamidine. The latter is now given frequently by the intramuscular route in Kala-azar (160).

V. SUMMARY AND CONCLUSION

The various studies reviewed have indicated that stilbamidine, pentamidine and the other aromatic diamidines have been effective as therapeutic agents in animal and human infections with *Leishmania* and *Trypanosomes*. Toxicity has been troublesome but not of such severity as to preclude rather extensive human trials. Despite the variations observed when *Trypanosomiasis* and *Leishmaniasis* are treated in different geographic areas, the value of the diamidines for the control of these infections has been evident. There is little doubt that in the management of cases resistant to antimony therapy, the diamidines are a distinct aid. Although stilbamidine appears to be somewhat the more effective drug, its neurotoxic properties preclude widespread use in the treatment of the milder *Leishmanial* infections (Indian). Pentamidine is the drug of choice for the therapy of such infections. Stilbamidine appears to be as effective as the older pentavalent arsenicals when used in the early stage of "African" *Trypanosomiasis*.

The general toxicity of the principle diamidines has not been fully determined. It appears that with the variable dosages employed thus far no serious visceral toxicity has been observed. Nevertheless, because of the hepatotoxic and nephrotoxic action of large doses in animals, caution is necessary in extending this form of therapy to patients suffering from renal or hepatic impairment. Stilbamidine alone has been associated with a late toxic neuropathy.

The chemotherapeutic application of the diamidines may be quite widespread. In addition to the *Trypanosome* and *Leishmanial* infections which have been favorably influenced by these drugs, plasmodial and babesial infection in mammals have responded (129). The growth of many bacteria is inhibited and fragmentary evidence has been noted that the proliferation of neoplastic cells is impaired by the diamidines. Perhaps most intriguing for those interested in the "mechanism of action" has been the appearance of granules in the cytoplasm of myeloma cells following therapy with stilbamidine. These granules apparently contain ribonucleic acid and stilbamidine. The importance of nucleic acid metabolism in neoplastic cells and the formation of these granules only in patients whose protein metabolism has undergone marked alteration have presented a problem worthy of further intensive investigation. Despite the significant morphological changes observed in the myeloma cells in the course of stilbamidine therapy, there is no indication that the course of the disease has been altered with this form of therapy.

It is of interest to note, however, that the cytoplasmic granules have been observed to persist in the myeloma cells for periods up to six months after treatment with stilbamidine has been discontinued. Myeloma cells containing the

stilbamidine-ribosenucleic acid granules in their cytoplasm have thus probably not undergone division during this period. This observation suggests that the multiplication of these tumor cells has been arrested to some extent.

The lack of an adequate method for the determination of pentamidine and the other non fluorescent diamidines has hampered investigations with these compounds. Stilbamidine has differed from the other diamidines because of its physico-chemical properties such as fluorescence, resonance, isomerism and adsorption. In addition it differs in its biologic activity with respect to protein denaturation and formation of protein and nucleic acid complexes.

Most of the discoveries in the chemotherapy of disease have been achieved through empiric trial. However, the importance of understanding the "mechanism of action" of such compounds has been repeatedly stressed in the hope that wider and more successful application might be attained and newer agents developed (130). The many drugs which are known to be biologically active due to their non-selective toxic action on most living cells have usually been classified as protoplasmic poisons rather than chemotherapeutic agents. The selective activity required for the latter has implied a differential effect more favorable to the host. The latter has been achieved with many substances, because of inactivation of specific enzyme functions, formation of unionized complexes with specific substituents of the invading parasite, or utilization or combination with necessary substrates required for proliferation, maintenance, etc. (56). It is evident that the chemical composition, metabolic activity, nutritional requirements and physico-chemical tolerances of both host and parasite will determine the efficiency and usefulness of a therapeutic agent. When the foreign cell is derived from the host cells, as in a neoplasm, these differential features may be very small indeed. It is clearly recognized that the host-drug relationship is as important as the host-parasite or parasite-drug relationships.

The diamidines have been shown to inhibit the growth of bacteria, protozoa, fungi and neoplastic cells. In many instances this inhibition has been followed by some irreversible alterations associated with death. These effects have been achieved at concentrations of the drugs at times far below those found toxic to experimental animals or man. Stilbamidine, together with the other diamidines, phenamidine, propamidine, and pentamidine, possesses non-selective toxic properties similar to many cationic detergents. However, the property of displacing basic proteins from combination with nucleic acids and the denaturation of proteins and possibly nucleic acid polymers places stilbamidine in a position of some importance in the search for new chemotherapeutic agents. Further investigation of compounds of similar structure and properties may reveal a new type of therapeutic agent effective against viral infections and neoplastic growth (58).

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CLINICAL FEATURES AND PATHOGENESIS OF TROPICAL SPRUE

OBSERVATIONS ON A SERIES OF CASES AMONG ITALIAN PRISONERS OF WAR IN INDIA*

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INTRODUCTION

This study presents clinical observations made over a period of almost three years on a large number of cases of tropical sprue occurring among Italian prisoners of war in a concentration camp in India. It is regretted that inadequate laboratory facilities and war time restrictions limited the investigation. Because of the unusual opportunity to follow the disease among a large number of individuals living under similar conditions of climate, housing and diet, interesting observations were obtained on the incidence, pathogenesis and clinical picture of the syndrome. However, they do not represent an isolated study. Several reports have appeared recently on sprue-like conditions occurring among British and Indian troops in the Indian war theatre. These syndromes, described under several names by the different authors, i.e., tropical sprue, hill diarrhea, nutritional diarrhea, present a rather uniform clinical picture with

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minor variations which are probably due to the differences in the race of the patients studied by the individual observers and to the area from which the condition was reported. Their characteristics also appear similar to those described in this study. Hence, an attempt will be made to correlate our findings with those previously reported and to show how these variations may modify existing ideas regarding the clinical picture, diagnosis and pathogenesis of sprue.

GENERAL OBSERVATIONS

Between March and June 1942, 12,500 Italian prisoners of war were transferred from various stations in India to a camp in Yol-Kangra Valley, where they remained until the beginning of 1946. The observations reported in this study were terminated in April 1945 because of the repatriation of a great part of the medical personnel and patients.

The camp was situated at an altitude of approximately 4,000 feet, in the foot hills of the Himalaya mountains. The humidity was high, especially during the rainy season (Table 1). The winter season, from December to February, was usually cold and rainy, followed by a period of two months of dry sunny weather with cool northwest winds. May and June were hot and dry. From the end of June until the end of September the valley had a long severe rainy season which was followed in October and November by cool sunny days.

The prisoners of the camp lived in wooden barracks provided with adequate ventilation. Water was obtained from surface springs and collected in large cement tanks through a pipe line five miles long. It was filtered and chlorinated before it was distributed to the camp. The bacteriologic examination was satisfactory. It was classified chemically as an oligomineral water of superficial spring source not containing mica or silicon in excess. The food issued to the prisoners will be considered later in the discussion of the clinical data.

Morbidity data were collected during the entire period of observation. It had been planned to integrate them with those obtained from a systematic survey of the morbidity among the civilian population of the valley, but this was prevented by several circumstances. However, considerable information was found to be available in the work of Megaw and Gupta (75) who had carried out a complete study of the epidemiology and morbidity of the area in 1927. They reported a high incidence of tick typhus, "hill diarrhea" (which is similar to the syndrome reported in this paper), benign tertian malaria, bacillary dysentery and goitre of the parenchymatous non-functioning type. Their findings were somewhat different from those of the survey we carried out among British and Indian troops and Italian prisoners of war stationed in the area, as would have been expected because of the differences of race, diet, habits, etc. During the three years of our observation only 9 cases of tick typhus (5 Indian, 3 British and 1 Italian) were diagnosed. After one year of residence in the area a large number of Italian prisoners developed goitre of the parenchymatous non-functioning type and the number of such cases increased progressively even after salt fortified with iodine was obtained for kitchen use. The possible relationship of this condition to sprue will be discussed later.

Morbidity data of the Italian prisoners of war are given in Table 2. Bacillary

TABLE 1

Climatic characteristics of Kangra Valley (period 1941-1944)

	JAN- UARY	FEB- RUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEP- TEM- BER	OCTO- BER	NO- VEM- BER	DE- CEM- BER
Humidity (per cent)												
Year 1941.	81	87	01	80	75	04	87	89	84	81	68	69
Year 1942	87	95	79	89	72	89	92	93	02	75	72	75
Year 1943	86	92	90	01	91	90	90	92	00	80	82	85
Year 1944 .	03	93	78	88	60	67	93	90	83	67	63	65
Precipitation (in cm)												
Year 1941 .	9.5	3.0	5.5	0.3	7.4	30.3	57.0	07.2	12.2	6.7	0.8	5.7
Year 1942	12.3	18.2	4.2	9.9	5.7	16.3	01.1	153.0	65.3	1.3	0.5	16.6
Year 1943	27.8	2.3	8.3	10.8	7.8	15.5	107.7	222.0	33.1	0	0.1	1.9
Year 1944	15.3	14.7	18.3	6.1	1.0	13.4	113.1	110.0	24.3	0.1	*	*
High and low temperatures (Fahrenheit)												
Year 1941												
High	70.2	72.4	84.2	87.8	106.0	110.2	102.6	94.0	87.8	86.0	82.0	70.2
Low	32.0	40.2	48.2	48.0	70.2	74.6	76.0	72.4	64.2	62.4	58.0	50.0
Year 1942												
High	74.0	79.4	82.2	90.0	115.0	111.8	108.2	94.2	94.0	00.0	82.2	68.0
Low	38.8	42.0	37.6	45.0	68.0	72.4	63.0	66.0	58.2	54.8	54.2	46.2
Year 1943												
High	72.4	78.2	70.4	91.8	107.8	106.0	100.0	91.8	90.2	90.0	84.2	72.4
Low	43.0	46.2	46.2	53.2	68.0	72.4	70.2	70.2	68.2	60.0	53.8	52.0
Year 1944												
High	72.8	80.0	94.0	102.0	108.2	102.0	96.2	88.0	84.2	84.2	82.2	71.2
Low.	35.8	46.2	46.2	48.8	59.8	53.2	64.2	62.2	53.8	51.8	50.0	37.6

* Data not available.

TABLE 2

Incidence per thousand of certain common diseases among Italian prisoners of war in Kangra Valley

	YEAR		
	1942	1943	1944
Malaria (new infestations) . .	4.5	2.7	1.2
Bacillary dysentery . . .	12.9	14.12	14.8
Amoebic dysentery* . . .	8.1	4.7	4.12
Renal calculosis . . .	8.7	9.2	11.10
Acute appendicitis	7.2	6.3	2.0
Goitre . . .	0	2.04	6.34

* Including relapses

dysentery and benign tertian malaria as well as renal calculus and acute appendicitis, whose high incidence remains unexplained, were among the most common causes of hospital admission. No data are given for British and Indian troops as their units in the area were changed too often to permit collection of significant information.

The nutritional status of the prisoners stationed in Yol was carefully followed especially after the report of the increasing number of cases of sprue. Repeated surveys were carried out every six months. The chief complaint among the prisoners was that of a slight but continuous loss of weight probably caused by dietary inadequacy although no signs of starvation or of specific deficiencies were observed in any patient free from sprue. Hematologic data from 100 individuals selected at random and in good health revealed a mild normocytic anemia (average figures: red blood count 4,310,000; Hb gm. 13.38%; volume of packed red cells 41.4%; color index 0.89).

CLINICAL FEATURES

1. *Epidemiologic Data.* The first cases of tropical sprue were diagnosed in September 1942, shortly after the end of the rainy season. From then until the end of April 1945, a total of 1069 cases of various clinical types and severity were encountered. Of these, 268, the most seriously ill, were admitted to the hospital while 810 were treated without leaving camp since adequate medical facilities were available there. The length of time that the prisoners were resident in the area bore a direct relationship to the number of new cases for a progressive increase was noted through the second and third year. On the other hand, the incidence of the disease during each year was clearly related to the atmospheric conditions for most of the cases occurred during or immediately after the rainy season, as shown in Table 3 and Figure 1. The incidence according to age group is shown in Table 4. The group from 25 to 35 years of age showed a relatively greater number of new cases and relapses. Although the greatest number of cases occurred in young prisoners, the more serious ones were found in the oldest age group in which 80% of the patients who died from the disease were between 50 and 55 years of age.

One or more relapses, usually during or immediately following the rainy season, were observed in 134 cases (12%). Death occurred in only 5 cases and in each it was attributable to aplastic anemia. This figure agrees with that given by other authors for groups of cases observed during a comparable period of time. Manson-Bahr and Willoughby (72) in a series of 200 patients found a mortality of 1.5%; Keele and Bound (55) in a series of 600 cases found 0.4%; Leishman (63) found about 1% mortality in an unspecified number of cases.

2. *History and Symptoms.* The past history of the patients was not contributory. 3% of them gave a previous history of malaria; 6% had suffered from acute bacillary dysentery during the year immediately proceeding the onset of sprue, 0.7% had chronic amebic dysentery, and 4% gave a history of syphilis (two individuals had a positive Wassermann test while showing active symptoms of sprue); and only one patient gave a history of peptic ulcer, although

the incidence of this disease among the prisoners in Yol was 4% over a period of three years. It was concluded therefore, that no condition appeared to have a direct bearing on the onset of sprue in these patients, although it was the general impression that any previous illness favored the development of the disease.

The patients' recent history was rather uniform. They usually reported to the hospital complaining of a severe, uncontrollable diarrhea, accompanied by distressing meteorism and often by a feeling of abdominal distension becoming especially unpleasant during the night. Other symptoms were sudden and

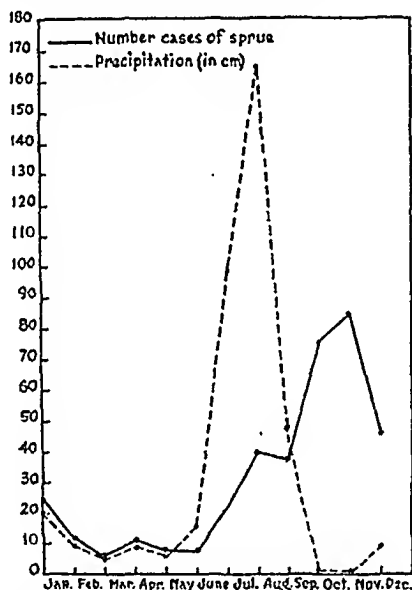


FIG. 1. INCIDENCE OF NEW CASES OF SPRUE PER MONTH DURING THE PERIOD 1942-1945 IN RELATION TO PRECIPITATION

The figures are the average for the three year period

severe asthenia, extreme irritability and nervousness, and tenesmus. If proper treatment was not instituted there appeared dysphagia, paresthesias, muscular cramps and vertigo. A few patients, who sought medical advice only after severe macrocytic anemia had set in, were bedridden. Many patients experienced difficulty in feeding themselves because of the dysphagia while others purposely avoided food to escape the extremely distressing meteorism following meals.

The classification of sprue into stages or clinical types is difficult because the symptomatology of the disease is inconstant owing to the varying frequency

and severity of the deficiency symptoms and to their different times of appearance during the disease. However, for purposes of description and on the assumption, which will be substantiated in the course of the paper, that onset and progression of sprue are related closely to the extension and severity of the intestinal malabsorption, the course of the syndrome has been divided into three stages. The first stage is characterized by fatty diarrhea and other early signs of intestinal malabsorption (nausea, anorexia, asthenia, etc.); the second stage is a multiple deficiency picture complicating the original symptomatology; the third stage is characterized by a complex deficiency syndrome dominated by a hyperchromic macrocytic anemia and presents all the symptoms which are usually considered typical of the full blown picture of tropical sprue. The proposed classification of the syndrome into stages and the incidence of the various symptoms in each stage are presented in Table 5.

First Stage. Diarrhea was the initial symptom complained of by all patients who reported for treatment. The nature of the diarrhea was obtained mainly from the patient's description as very few of them sought medical advice early in the course of the disease. The diarrhea was of sudden onset in 73%, it was uncontrollable ("explosive") and from 5 to 15 stools were passed daily from the onset of the disease. In the remaining cases the diarrhea was less severe with few movements in the early morning but as the disease progressed the diarrhea suddenly became more severe. The characteristics of the feces were not constant: in 46% of the cases they appeared semisolid (average weight being 100 to 200 gm per movement), frothy, pale and containing undigested food and their passage was usually accompanied by distressing tenesmus. In 17% the feces were pale and watery with a large amount of undigested food. In the remaining 21% the feces were of a mixed character. In each patient the feces were offensive and gave an acid reaction. In only 15% did the feces conform to the classical description given for sprue (pale, fatty, bulky and frothy).

The rhythm of the diarrhea during the day was not uniform. The movements occurred in the early hours of the evening in 9%, in the early morning hours in 49%, during the afternoon hours in 12% and shortly after meals in 29% of the cases. The onset of diarrhea in each patient was accompanied by extreme *asthenia* which was out of proportion to the number of movements or to the severity of the diarrhea. Indigestion was also present and all patients complained of *meteorism* which was especially distressing at night. It was improved temporarily by the passage of flatus or feces. *Anorexia*, *nausea* (6%) and *vomiting* (0.4%) were rather uncommon. The loss of weight was rapid and severe (14 to 20 pounds in a period of 2 to 3 weeks). A mild continuous *fever* of a few days duration at the onset of the disease was recorded in 12% of the cases. The early occurrence of this symptom was surprising as it has been described rarely (36). It is usually present during the terminal stages of the disease (3), in late cases with an unfavorable prognosis (16), or during relapses (118). It is interesting to note that the course of the disease was particularly severe in a large number of the patients (78%) who presented fever as an initial symptom and that five of them developed aplastic anemia. Few laboratory data were

collected since there was only a limited number of patients in the initial stage of the disease at the time that they reported to the hospital or to their camp doctor. Blood counts revealed a mild hypochromic normocytic anemia and intestinal malabsorption appeared well established already because the oral glucose tolerance test (patients No. 502, 627, 972, Table 7) showed the typical flat curve and because the chemical analysis of fecal fat gave results identical to those obtained in the later stages of the disease (patients No. 627 and 972, Table 10). An average period of 2 to 3 weeks elapsed between the onset of the diarrhea

TABLE 3

Number of new cases of sprue per month occurring among Italian prisoners of war in Kangra Valley during the period March 1942-April 1945

	JAN- UARY	FEB- RUARY	MARCH	APRIL	MAY	JUNE	JULY	AUG- UST	SEP- TEM- BER	OCTO- BER	NO- VEM- BER	DE- CEM- BER
Year 1942									21	44	50	41
Year 1943	4	6	8	15	11	4	23	30	31	49	55	9
Year 1944	48	16	4	14	6	13	21	52	61	138	151	92
Year 1945	23	18	7	4								
Total	75	40	19	33	17	17	44	82	113	231	256	142

TABLE 4

Occurrence of fresh and relapsing cases of sprue per age group
(Percentage of all prisoners present in the camp per single age group is included)

AGE GROUP	PRISONERS IN EACH AGE GROUP	NEW CASES		RELAPSES	
		number	%	number	%
years	%				
18-25	12.10	69	6.46	6	4.48
26-30	23.19	352	32.87	40	29.86
31-35	27.01	402	37.61	62	46.26
36-40	11.72	124	11.61	18	13.43
41-45	9.43	98	9.18	5	3.73
46-50	8.54	16	1.51	1	0.75
51-55	6.99	8	0.76	2	1.49
over 55	1.02	0	0	0	0
Total . .		1069		134	

and associated symptoms and the time of appearance of the first signs of deficiency. In our patients the onset of the deficiency symptoms did not precede the onset of diarrhea.

Second Stage. Deficiency Symptoms. The majority of the patients were observed during this period, in which various deficiency symptoms due to a defect of intestinal absorption of nutrients and vitamins complicated the initial picture. Deficiency signs were first noticed from 15 to 20 days after the onset of diarrhea. Meanwhile, during this period of time, certain initial symptoms of

the syndrome underwent modification such as a diminution in the number of stools with the feces appearing more solid. When present, the fever, nausea and vomiting subsided. On the other hand, deterioration in the general condition of the patients continued as most of them lost between 30 and 45 pounds in 2 or 3 weeks and continued to suffer from severe asthenia. In this stage of the disease the patients usually complained of a severe burning pain in the mouth and retrosternal area during attempted feeding. There were paresthesias especially in the lower limbs in 17% of the cases and 27% experienced muscular cramps which were usually infrequent and mild but occasionally so severe as to interfere with rest and sleep.

The objective findings were typical. *Glossitis* was present in 90%. At first the edges and tip of the upper surface of the tongue appeared reddened. A few days later the areas appeared de-epithelized, reddened and covered in spots by small aphtae. In several cases the same lesions involved other parts of the oral cavity such as the lower surface of the tongue (13%), the soft palate and uvula (4%) and the buccal mucosa (7%). In a few patients other mucosae also presented dystrophic lesions: *angular stomatitis*, with or without *cheilosis*, was recorded in 27, while proctoscopic examination of the patients complaining of severe tenesmus (29%) revealed a reddened and congested rectal mucosa. Other deficiency symptoms usually appeared slightly later in the course of the disease. Several patients presented *exfoliative dermatitis* limited to the scrotum (25%) or to the thighs, abdomen, buttocks and dorsal surface of the hands (9%). These lesions usually consisted of small, irregular, shiny plaques which tended to desquamate and were accompanied by moderate hyperkeratosis follicularis. Muddy brownish spots of cutaneous hyperpigmentations were present on the face, hands, feet (dorsal surface), abdomen and buttocks in 33%. No spots of hyperpigmentation were found on examination of the mucosae. In 21% the distal inch of the hair changed into a lighter color (brownish in dark-haired individuals) and in most cases the body hair was likewise involved.

The abdomen appeared distended due to meteorism. *Hypotension* was a constant finding. In 92% the average systolic blood pressure was 108 mm. Hg., the diastolic 64 mm. Moderate hypochromic normocytic anemia was usually observed in this stage of the disease, which gradually changed to a hyperchromic type in those cases who did not respond to treatment. Microscopic examination of the feces of these patients were carried out routinely and no significant findings were noted with the exception of the presence of cysts of *Entamoeba histolytica* in 0.7% of the cases (many of the patients had lived for some time in East Africa and in the plains of India). A scanty exudate was noted in a few cases and *Bacillus paratyphosus Flexneri* was cultured.

Few biochemical findings confirmed the defect of intestinal absorption already found in the first stage of the disease. The oral glucose tolerance test gave a typical flat curve in five patients (Nos. 12, 19, 182, 331, 903, Table 8). Studies of fat absorption and distribution of fecal fat (Nos. 19, 182, 331, 903, 1012, Table 10) gave similar results to those in the more advanced stages of the disease.

Third Stage. Hyperchromic Macrocytic Anemia. Hyperchromic macrocytic

anemia developed 4 to 5 weeks after the onset of diarrhea, which completed the typical full blown picture of tropical sprue in 153 patients (14%). Patients who reached this stage of the disease were among those who did not request medical care sooner or who did not respond to treatment. This figure gives a lower incidence of hyperchromic macrocytic anemia than that reported by earlier observers. Manson-Bahr and Willoughby (72) reported macrocytic anemia in 50% of their 200 cases, Charmichael-Low (17) in practically all of his 150 and Castle et al. (19) in 90% of their 92 selected cases. Other authors working in India at the time of our observations (21, 12, 55, 118, 33) also reported a higher incidence than we observed. This lack of agreement in the percentage of cases

TABLE 5

Classification and incidence of symptoms of the syndrome, divided by stages

	PER CENT
a) <i>Initial stage</i>	
Diarrhea	100
Dyspepsia	100
Nausea	5.8
Vomiting	0.5
Fever	12.10
b) <i>Stage of secondary nutritional deficiencies</i>	
Diarrhea	100
Glossitis	90.4
Angular stomatitis	27.22
Catarrhal proctitis	29.47
Exfoliative dermatitis	33.67
Pigmentations	32.70
Paresthesias	17.40
Muscular cramps	27.20
Hypotension	92.40
c) <i>Stage of fully developed picture of tropical sprue (in addition to diarrhea and deficiency symptoms)</i>	
Anemia (hyperchromic macrocytic)	14.4
Edema	10.2

showing hyperchromic macrocytic anemia may be explained by the difference of the severity of the disease at the time of observation. The onset of hyperchromic anemia altered the type and severity of the pre-existing symptoms. The number of stools was reduced to 4 or 5 a day and were early in the morning; meanwhile the feces gradually developed the typical characteristics that are described for sprue. Glossitis, stomatitis, proctitis, etc. of the second stage persisted with some diminution of dysphagia and tenesmus.

The patient in this stage presented a typical appearance. Asthenia, meteorism, vague but persistent abdominal pains usually made confinement to bed obligatory. In many cases the spotted hyperpigmentations offered a striking

contrast to the pallor of the skin, which was rough and dry. The musculature was hypotrophic and the subcutaneous fat almost non-existent. Most of the cases also presented edema of the face and extremities. The abdomen appeared distended and vermicular movements of the intestinal loops could be observed through the thin abdominal wall. No heart enlargement was found at physical and fluoroscopic examination even in cases with extreme anemia although a functional apical systolic murmur and hypotension were usually present. The liver size was within normal limits but the spleen was moderately enlarged in 24 individuals, 15 of whom had had repeated attacks of malaria. A few patients showed exaggerated tendon reflexes but as a rule the neurological examination was negative. Irritability, restlessness, inability to concentrate and loss of memory were frequent complaints. Hemorrhagic manifestations were present only in a single fatal case. In this patient cutaneous purpura and retinal hemorrhages were observed during the terminal stage of evolution to aplastic anemia (erythrocytes: 750,000, platelets: 22,700, tourniquet test strongly positive). Post-mortem examination revealed diffuse punctate hemorrhages, especially of the gastric and intestinal mucosa and thrombosis of the central retinal vein in the right eye. In 45 other patients in this stage of the disease the clotting and bleeding times, tests for capillary fragility and the prothrombin time were found normal.

Studies of the hematological picture were performed in all the 153 cases which demonstrated the hyperchromic macrocytic type of the anemia. The average hemoglobin (Hb) value, determined by Sahli's method (100% of the scale is equal to 17.3 gm. Hb per 100 ml. of blood) was 11.16 gm. The range of the hemoglobin values was 9.51 to 12.11 gm; the number of erythrocytes averaged 2,870,000 per mm³ with individual values from 1,700,000 to 3,600,000; the average color index was 1.13 with a range of 1.06 to 0.82; the average volume of packed red cells (Wintrobe) was 35.4%. Other data for the determination of the type of the anemia were calculated from the preceding figures and have been tabulated below. The values shown in brackets were calculated from the results obtained in 100 normal individuals.

Mean corpuscular Hb, $\gamma\gamma$	33.8	(20.04)
Mean corpuscular volume, $c\mu$	123.34	(94.06)
Volume Index (84).....	1.47	(1.02)
Mean corpuscular Hb concentration, %.....	31.5	(37.90)
Saturation index.....	0.92	(1.15)

Microscopic examination of the peripheral blood showed anisocytosis, poikilocytosis and polychromatophilia while in a few severe cases erythroblasts and normoblasts were observed also. The number of reticulocytes averaged 1.8% before treatment was started. Interesting changes were likewise noted in the leucocytes. In 32% of the cases the white cell count was slightly higher (average 8,600) than the figure for normal subjects (average of 8,000 in 100 normal individuals) while the remaining 68% of the cases showed a moderate leucopenia (average 5,650). The differential count was normal in the majority of the cases; there was, however, a moderate lymphocytosis in 24% of the subjects while 12%

presented eosinophilia. The platelet count was normal (average number 272,000). Sternal bone marrow biopsies were performed in 25 cases. The smears were stained with May Grunwald-Giemsa's and 500 cells were counted in each case. The results are reported in Table 6. With the exception of cases No. 67, 937 and 987 all showed the typical erythroblastic arrest indistinguishable from that found in pernicious anemia. It will be noted that biopsies

TABLE 6

Sternal bone marrow in 25 untreated cases of tropical sprue (count on 500 cells)

CASE NO.	ME	E. ER.	L. ER.	NORM.	GRAN. S.	LYMP. S.	PLAS.	MEGAK.	NON C.C.
	%	%	%	%	%	%	%	%	%
27	8.0	7.2	10.0	17.8	52.0	1.4	1.2	0.4	2.0
56	18.2	13.4	12.6	11.0	34.0	8.0	0.4	0.6	1.8
67	2.2	6.4	4.8	7.2	69.8	8.8	0.2	0.4	0.2
194	14.2	10.6	12.4	6.0	51.2	2.4	3.0	0.2	0.0
196	9.6	8.4	5.6	14.0	54.0	4.8	2.4	0.0	1.2
303	21.4	10.6	14.0	9.0	31.0	11.8	0.4	0.4	0.8
352	12.8	14.0	9.0	8.4	43.6	11.6	0.4	0.2	0.0
472	5.4	5.4	10.0	15.4	52.2	8.4	2.2	0.0	1.0
524	25.0	21.0	5.0	6.0	29.0	10.0	0.0	0.6	3.4
601	27.2	18.0	14.0	7.4	23.8	7.2	1.0	0.4	1.0
701	7.0	11.8	8.2	10.8	52.2	6.2	1.2	0.0	2.0
792	18.0	10.0	12.4	14.0	32.8	10.6	1.0	0.2	1.0
801	13.0	14.2	11.8	17.0	37.4	4.0	1.4	0.0	1.2
820	10.0	5.0	8.6	12.4	58.8	3.4	0.6	0.4	0.2
847	26.4	21.0	9.0	6.6	32.4	3.2	0.8	0.2	0.4
899	3.0	8.0	7.8	16.2	43.4	20.6	0.4	0.6	0.0
907	21.8	13.0	12.4	4.6	41.8	4.8	1.0	0.0	0.6
937	0.8	7.6	8.8	17.4	44.8	17.0	2.2	0.6	0.8
954	17.4	12.8	10.8	11.0	42.0	3.4	2.0	0.2	0.4
959	12.2	9.8	10.6	16.6	39.0	10.0	1.2	0.4	0.2
975	7.2	12.0	7.8	9.0	48.6	14.8	0.2	0.2	0.2
978	24.2	14.0	9.4	6.2	38.6	7.2	0.4	0.0	0.0
987	1.8	3.6	0.4	5.4	73.6	8.2	0.0	0.4	0.6
1027	19.6	18.2	12.0	11.6	28.8	7.6	0.8	0.0	0.8
1062	10.4	8.2	8.0	11.6	54.4	5.0	1.6	0.4	0.4
Average...	13.60	12.45	9.75	9.59	41.06	8.11	1.14	0.39	0.91

Me., Megaloblasts; E. Er., Early erythroblasts; L. Er., Late erythroblasts; Norm., Normoblasts; Gran. S., Granulocytic series; Lymph. S., Lymphocytic series; Plas., Plasma cells; MegaK., Megakaryocytes; Non C. C., Non classifiable cells.

of the sternal marrow performed in three of these patients when in the second stage of the disease had shown only a moderate hyperplasia of the normoblastic type. These results closely agree with those reported by other authors (57, 90, 111). Tests for red blood cell fragility were discontinued after obtaining normal values in 30 cases. The Takata-Ara test was done in 98 cases with negative results in 91. A positive reaction was obtained in the serum of one patient with a severe anemia who did not respond to treatment and later developed aplastic

contrast to the pallor of the skin, which was rough and dry. The musculature was hypotrophic and the subcutaneous fat almost non-existent. Most of the cases also presented edema of the face and extremities. The abdomen appeared distended and vermicular movements of the intestinal loops could be observed through the thin abdominal wall. No heart enlargement was found at physical and fluoroscopic examination even in cases with extreme anemia although functional apical systolic murmur and hypotension were usually present. Liver size was within normal limits but the spleen was moderately enlarged in individuals, 15 of whom had had repeated attacks of malaria. A few showed exaggerated tendon reflexes but as a rule the neurological examination was negative. Irritability, restlessness, inability to concentrate and memory were frequent complaints. Hemorrhagic manifestations were seen only in a single fatal case. In this patient cutaneous purpura and hemorrhages were observed during the terminal stage of evolutionary anemia (erythrocytes: 750,000, platelets: 22,700, tourniquet test positive). Post-mortem examination revealed diffuse punctate hemorrhages especially of the gastric and intestinal mucosa and thrombosis of a retinal vein in the right eye. In 45 other patients in this stage of anemia clotting and bleeding times, tests for capillary fragility and the Rous test were found normal.

Studies of the hematological picture were performed in all cases and demonstrated the hyperchromic macrocytic type of the anemia. The hemoglobin (Hb) value, determined by Sahli's method (normal value equal to 17.3 gm. Hb per 100 ml. of blood) was 11.16 gm. in the average; the hemoglobin values were 9.51 to 12.11 gm; the number of erythrocytes was 2,870,000 per mm³ with individual values from 1,700,000 to 4,000,000; the average color index was 1.13 with a range of 1.06 to 1.25; the packed red cells (Wintrobe) was 35.4%. Other data of the type of the anemia were calculated from the preceding values and are tabulated below. The values shown in brackets were obtained in 100 normal individuals.

Mean corpuscular Hb, $\gamma\gamma$
Mean corpuscular volume, $c\mu$
Volume Index (84).....
Mean corpuscular Hb concentration, %.....
Saturation index.....

Microscopic examination of the peripheral blood smear showed poikilocytosis and polychromatophilia while in normoblasts were observed also. The reticulocyte count before treatment was started. Interleukocytosis. In 32% of the cases the number of leukocytes was less than 8,600) than the figure for normal individuals) while the remaining 68% (average 5,650). The differential count showed that there was, however, a moderate leucopenia.

TABLE 7—*Concluded*

CASE NO.	CALCIUM	PHOSPHORUS	GLUCOSE	CHOLESTEROL	SEDIMENTATION RATE	NON-PROTEIN NITROGEN	BILIRUBIN
	mg. per 100 ml.	mg. per 100 ml.	mg. per 100 ml.	mg. per 100 ml.	mm. per hr.	mg. per 100 ml.	mg. % per 100 ml.
913	.		98.4		8.2	37.2	0.47
937	8.9	3.9	93.7	134.7	5.2	28.5	0.38
942					3.2	27.8	0.32
954	9.2	4.7	89.3	149.0	6.5	24.0	0.40
957			94.2		7.0	26.9	0.43
959	8.8	3.9	98.3	150.8		25.5	0.61
964					10.2	41.5	
975	7.0	4.0	97.0	151.4	4.0	29.8	0.41
978	8.4	4.0	90.0	123.6	7.4	23.2	0.27
981			97.6		5.8	29.4	
987	7.7	5.1	88.5	142.0	10.0	30.1	0.36
1011			95.2		12.8	30.9	0.30
1027	7.6	3.8	94.0	143.0	7.8	26.1	0.39
1054					13.0	41.2	0.34
1062	9.6	3.1	93.2	147.4	9.0	35.0	0.39
Total average. . .	8.36	4.06	92.72	141.18	7.87	29.83	0.39

Serum calcium level was determined with the method of Roo and Kahn (93). Serum phosphorus with the method of Bell and Doisy as modified by Briggs (13). Fasting blood sugar level with the method of Hagedorn and Jensen (42). Serum cholesterol with the method of Bloor et al. (11). Serum bilirubin with a modification of the method of Van den Bergh. Blood sedimentation rate of citrated blood was read after one hour according to Westergren (121).

anemia. In six patients a weak positive reaction was obtained; all these subjects presented a severe picture of macrocytic anemia and one had been suffering from a recent attack of malaria.

Several chemical determinations of blood constituents were made in a limited number of cases. The results are summarized in Table 7. The serum calcium levels were always below normal, while the serum phosphorus values were within normal limits. Similar results were obtained in a few patients just entering the second stage of the disease. These findings agree with those reported by Fairley (36) who, in his series of 70 cases, found that hypocalcemia was constant and paralleled the degree of steatorrhea. These data suggest that an abnormally high fixation of the ingested calcium by fatty acids and not a specific defect of absorption is responsible for the hypocalcemia of sprue. The degree of hypocalcemia was not related to muscular cramps.

The non-protein nitrogen level was within normal limits in the majority of cases, its limited variations being more closely related to age or pathological conditions present than to sprue itself. Serum cholesterol and serum bilirubin values were normal. This observation may be contrasted with Snell's finding (99) of a low cholesterol level proportional to the severity of the disease in 12

cases of non-tropical sprue but agrees with the observations of Suarez et al. (111) in a series of 16 cases of tropical sprue.

Fasting blood sugar levels were consistently lower than normal, in agreement with those given by Fairley and Mackie (37) (average 84 mg. % in 17 cases). The high values given by Serra (97) (average 101 mg. % in 48 cases of tropical

TABLE 8

*Blood sugar concentrations during the oral glucose tolerance tests in 24 cases of tropical sprue**

Concentrations are expressed as mg. per 100 ml.

CASE NO.	TIME				
	0 min.	30 min.	60 min.	90 min.	120 min.
Patients in the first or second stage of the disease					
12	94.3	112.7	128.4	108.3	
19	101.4	126.0	142.7	117.2	94.0
182	87.5	104.3	119.8		88.2
331	92.7	116.8	132.3	103.6	90.5
502	91.8	112.2	127.5	99.2	87.6
627	89.2	111.7	128.6	102.9	92.6
903	87.8	110.2	127.2	97.7	91.1
972	97.5	124.9	139.1	115.8	93.9
Patients in the third stage of the disease					
49	102.4	121.3	135.4	116.9	
67	85.2	100.7	112.9		84.4
76	87.9	94.5	101.7	91.0	86.1
152	86.4	104.2	117.3	96.2	87.7
189	110.2	117.0	129.1	112.7	103.7
196	88.0	106.1	114.5	99.4	84.2
352	101.4	121.9	135.4	115.3	92.0
524	89.7	114.8	131.4	107.5	90.8
618	97.4	112.7	120.2		89.0
701	94.5	109.4	118.0	106.0	92.4
727	97.2	106.5	114.1	101.7	94.2
792	92.9	113.6	122.8	107.2	88.9
899	89.7	101.2	109.7	96.9	
954	89.3	104.0	115.2	98.7	87.3
987	88.5	107.2	122.2	101.2	85.1
1062	93.2	108.8	119.3	104.8	90.7
Average.....	92.75	110.93	123.56	104.77	90.20

*The patients received 1 gm. of glucose per kg. weight at the beginning of the test.

sprue) are difficult to explain when one considers the impairment of carbohydrate absorption usually present. In 7 cases in the first or second stage and in 16 cases in the third stage of the disease the oral glucose tolerance curve gave a uniform response with a moderate and transitory hyperglycemia not followed by any hypoglycemic terminal reaction (Table 8). No curves with a normal but

delayed rise in blood glucose level, as described by Fairley (36) were observed. No intravenous glucose tolerance tests were done. The blood sedimentation rate was within normal limits, even in patients with pronounced anemia. Moderate urobilinuria was found in all 153 cases in the third stage and in 114 in the second stage of the disease. There were no other abnormal urinary findings.

The results of gastric analysis in 97 cases using the Cade and Milhaud's test meal (15) are shown in Table 9 and Figure 2. Samples of gastric juice were collected every 15 minutes for 2 hours. The free HCl content was titrated with

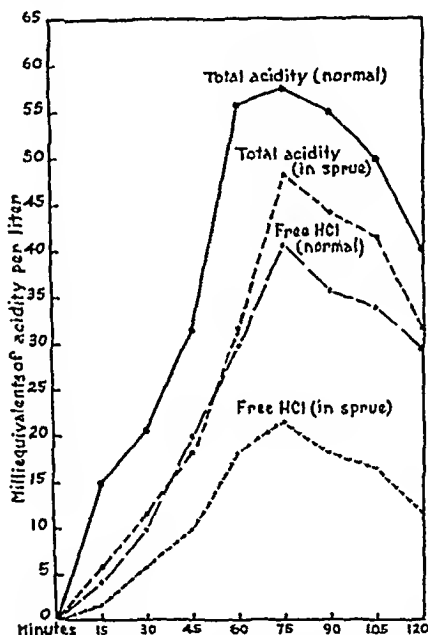


FIG. 2. GASTRIC ACIDITY IN NORMAL SUBJECTS AND IN PATIENTS WITH TROPICAL SPRUE

Topfer's reagent. Most of the cases showed moderate hypochlorhydria; six cases presented achlorhydria which responded to histamine. In one chronic case there was a true achlorhydria. Two patients showed hyperchlorhydria (one presented active symptoms of duodenal ulcer at the time of examination). No lactic acid was found in any specimen. Our findings are different from those presented by other investigators (55, 118, 33, 36) who found normal acidity curves. This divergence may possibly be explained by a difference in the severity of condition of the patients studied or type of test meal employed. For example, Castle et al. (19) found relative or absolute achlorhydria after oral

alcohol stimulation in 38% of their 65 cases of severe type. No determinations of pepsin activity were done.

✓ Duodenal intubation was performed in 47 cases, 25 in the second and 22 in the third stage of the disease and the various bile fractions were studied with Meltzer-Lyon's technique (76). No pathological changes were found in any instance. Microscopic examination of the sediment and cultures were negative. No studies of pancreatic digestion were carried out because laboratory facilities were inadequate.

— It had been planned originally to give particular attention to fat absorption and the distribution of fecal fat. However, in view of practical reasons, such studies had to be limited to twenty cases, 7 in the first or second and 13 in the third stage of the disease. The experimental conditions were carefully standardized. For four consecutive days all the patients were placed on Schmidt and Strasburger's diet (95) which contains 80 gm of fat. The diet was prepared in the hospital kitchen and the fat content was calculated from the food tables of McCance and Widdowson (74). Some patients did not tolerate the high fat content of the diet very well but even so the diet was not altered to fit the individual taste for it was feared that different amounts of fat would modify the results by varying the emptying time of the stomach. The stools were collected in the second, third and fourth days of experiment. 50 ml. of 5% formalin were added as a preservative to the pooled specimen from each patient. Each specimen was then dried and weighed before determining the total fat, fatty acids and soap content by the method of Saxon, as modified by Holt et al. (50). From these results the ratio of split to unsplit fecal fat and the ratio of soaps to split fecal fat was calculated.

The average daily weights of the stools of the patients were much higher than those of normal individuals. The fat content of the stools was also found to be increased, especially during the third stage of the disease, but the ratio of split to unsplit fat was not much higher than normal (3.87 as compared to the average normal value of 3.15 in the control cases). Despite the slight acid reaction of the feces which had the approximate pH of 6.7 when determined with indicator strips, it was found that most of the split fat fraction consisted of soaps. The ratio of soaps to split fat was 0.64 as compared with a normal ratio of 0.51. In conclusion, the results confirmed the typical steatorrhea with a high ratio of split to unsplit fecal fat usually described in tropical sprue. They also revealed that a large amount of split fat was present in the feces in the form of soaps. The standardization of our experimental conditions permitted a relatively accurate determination of the percentage of fats absorbed. This was calculated from the amount of fat ingested on the Schmidt and Strasburger's diet and the amount of fat eliminated in the feces according to the formula:

$$\text{Percentage of fat absorption} = \frac{\text{Dietary fat} - \text{Fecal fat}}{\text{Dietary fat}} \times 100$$

The average results are presented in Table 10. Under the experimental conditions normal subjects absorbed approximately 90% of the dietary fat while the patients with sprue absorbed only 66% of the ingested fat.

X-ray examination of the long bones in 12 cases revealed no pathological findings.

3. *Course of the Disease.* The course of the disease was generally favorable when therapy was instituted early. Only 47 patients (5%) in the first and second stages of the disease failed to respond to treatment and these, together with the 106 cases who were diagnosed in the third stage of the disease, represent the 153 patients who developed the hyperchromic macrocytic anemia.

During the three year period 112 patients suffered a relapse during or immediately after the rainy season; of these, 23 relapsed each year at the same time. Frequency of relapse was in direct proportion to the severity of the disease; therefore the patients suffering from macrocytic anemia had a higher incidence of relapses (78% as compared to 22% of the total number of cases). The onset of

TABLE 9
*Gastric acidity**
Concentrations are expressed as m.eq.

	STAGE OF DISEASE			
	Second	Third	Chronic	Relapse
Number of cases examined	32	46	9	10
True achlorhydria	0	0	1	0
False achlorhydria**	0	2	3	1
Hypochlorhydria	24	39	4	7
Normochlorhydria	7	4	1	2
Hyperchlorhydria	1***	1	0	0

* Values from 45 to 87 milliequivalents for total acidity and from 32 to 58 milliequivalents for free HCl were considered normal. They represent maximal and minimal values obtained in over 50 patients detained in the hospital in the same period of time and free from gastrointestinal symptoms.

** The cases of false achlorhydria include those which did not respond to the test meal originally but did so subsequently under histamine stimulation.

*** This patient was suffering from active duodenal ulcer at the time of examination.

relapse reproduced the picture found in the first stage of the disease with severe fatty diarrhea, anorexia and pronounced asthenia. No other symptoms developed, on account of the early treatment.

The disease ran a chronic course in 4 patients; each was older than 45 years of age. These subjects had a full blown picture of sprue in a well advanced stage. They did not respond to large doses of liver extract given parenterally or to blood transfusions. Their original clinical picture changed slowly. The number of daily stools was reduced but the daily weight of feces remained approximately the same; the stools gradually assumed the characteristics considered typical of tropical sprue. Anorexia, meteorism and asthenia persisted and in 2 cases became worse. The glossitis appeared modified after two or three months as the dorsal surface of the tongue appeared "geographic" with almost complete atrophy of the mucosa and hypertrophy of the underlying reticular tissue. Sigmoido-

scopic examination at the same time revealed greyish, diaphanous, atrophic mucosa scantily covered with mucus. Neither the blood picture nor the general condition of these patients responded to even the most extensive treatment. They have been observed since their repatriation to Italy and after two years they still presented active symptoms of the disease and required continuous treatment.

Five cases developed aplastic anemia. Their symptoms became steadily worse notwithstanding therapy. Their progressively severe anemia soon became critical and numerous blood transfusions were required. Because of difficulties encountered in obtaining donors the use of blood in these cases was irregular in regard to frequency and quantity and these patients finally succumbed to intercurrent respiratory infections. Hemorrhagic manifestations were conspicuous by their absence, even with a low platelet count, ranging from 37,000 to 92,000 in four cases before death. The remaining patient presented numerous petechiae shortly before death, while examination of the fundus oculi revealed diffuse hemorrhages and thrombosis of the inferior nasal and temporal branches of the right retinal vein. At this time the platelet count was 27,000. The peripheral blood and sternal bone marrow in these cases presented a typical picture of aplastic anemia. The condition of these patients prevented more frequent biopsies, so that no studies of the progressive changes in the characteristics of the bone marrow could be carried out.

4. *Pathologic Findings.* Autopsies were performed in the five fatal cases but little information was obtained. The mucosa of the gastrointestinal tract appeared uniformly pallid and atrophic. The wall of the intestine appeared thinner than normal. Bone marrow of the metaphyses and epiphyses of the femurs was gelatinous, that of the ribs and sternum was scanty and pale with the typical microscopic changes usually found in aplastic anemia. Specimens of different organs for histologic examination were sent to the Central Laboratory, India Command, in Poona, due to the lack of laboratory facilities locally. In all five cases the pathologist reported aplasia of the bone marrow and lesions of the gastrointestinal tract consisting of extensive atrophy of the superficial epithelium and of the epithelium of the villi, of edema of the submucosa and of the interfibrillar spaces and atrophy of the muscular layer. No other organs or tissues showed any specific alteration.

It appears, then, that these cases presented clear macro and microscopic intestinal lesions which probably accounted for the irreversible impairment of intestinal absorption. But, since the post-mortem examinations were carried out several hours after death in patients presenting an extreme degree of protracted anemia, we do not believe that the findings contribute to the solution of the controversy regarding the entity and significance of the inconstant macro and microscopic pathological findings in the intestinal tract of patients who succumbed to sprue or to its complications. We agree with Stannus (102) that post mortem findings may have little or no bearing on the functional derangements underlying the clinical symptoms; we also feel that endoscopic examinations during life are probably more important for the understanding of the pathology of the gastrointestinal tract in sprue. In cases with acute symptoms the gastric

mucosa has been observed to present patches of atrophy, purpuric spots, signs of inflammation, and edema. In the majority of the advanced cases gastroscopic examination showed a secondary type of atrophic gastritis (82, 47) and digital examination of the rectum showed obliteration of the mucosal folds (56).

TABLE 10
Studies on fecal fat distribution and fat absorption in tropical sprue*

CASE NO.	WEIGHT DRY FEELS	TOTAL FAT	TOTAL FAT				ABSORP- TION	S/U FAT RATIO	S/S FAT RATIO
			Split	F.A.	Soaps	Unsplit			
Patients in the first or second stage of the disease									
	lbs	%	%	%	%	%	%		
19	58.2	21.2	81.2	21.2	57.4	18.4	73.5	4.41	0.70
182	67.1	14.0	80.1	27.7	53.0	19.3	82.5	4.14	0.65
331	49.5	24.0	78.5	29.4	49.4	21.2	70.9	3.70	0.47
927	52.7	30.0	77.0	28.6	48.7	22.7	62.5	3.39	0.60
903	72.4	26.3	81.4	29.2	52.4	18.4	67.1	4.42	0.69
972	61.6	25.7	79.1	27.1	52.0	20.9	67.8	3.78	0.81
1012	54.9	30.1	81.7	29.3	52.6	18.1	51.8	4.45	0.56
Average...	59.48	25.31	79.80	27.93	52.21	19.80	68.4	4.02	0.63

Patients in the third stage of the disease

49	46.4	30.9	78.4	30.4	48.0	21.6	61.9	3.63	0.61
67	51.9	16.7	77.1	27.9	49.3	22.8	79.1	3.39	0.61
152	64.9	38.1	80.5	32.6	48.0	19.4	52.4	4.15	0.59
524	49.2	22.9	78.9	31.9	46.9	21.2	71.8	3.77	0.59
618	49.7	17.4	79.6	28.4	51.2	20.4	78.2	3.56	0.61
672	40.8	19.2	80.1	29.0	50.8	19.6	75.7	4.14	0.60
684	42.4	20.0	79.9	25.7	51.1	23.2	68.0	3.93	0.60
701	47.9	32.9	81.3	26.8	54.5	18.7	58.8	4.41	0.60
792	51.3	34.7	81.4	24.4	57.0	18.6	63.1	4.46	0.61
801	59.4	24.0	80.7	27.2	53.4	19.4	70.0	4.28	0.60
954	57.2	31.7	75.8	31.4	44.4	24.2	72.2	3.77	0.57
959	51.4	32.1	79.0	21.7	57.6	20.7	71.4	3.77	0.57
1062	40.9	30.4	78.1	20.9	58.3	21.8	67.1	3.57	0.58
Average..	50.00	27.4	79.04	23.76	55.42	21.55	67.1	3.77	0.58

Normals (5 examined). Average figures

34.00	9.2	75.79	37.35	55.75	21.55	67.1	3.77	0.58
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F. A.: Fatty Acids; S/U Fat Ratio: Soap/Fat Ratio

* Values given represent the average of two or three day period.

We were not able to perform the necessary studies for the acute, initial stage, the findings agree with the congested and covered findings for

set in, there were glassy, pinkish, irregular patches of atrophy. In the four chronic cases and in the five that developed aplastic anemia the mucosa appeared greyish in color, diaphanous and scantily covered by mucus. In the five fatal cases the atrophic lesions of the intestinal mucosa seen ante mortem were con-

TABLE 11

*Composition of the various diets used in the treatment of tropical sprue**

	DIET 1	DIET 2	DIET 3
Milk, gm.....	1,400**	900**	1,000
Fruit, fresh, gm.....	250 (juice)	250	250
Liver, gm.....	250***	180	180
Vegemite****, gm.....	50	50	50
White of eggs, gm.....	50		
Tomatoes, gm.....	50 (juice)	50 (juice)	50 (juice)
Mutton, gm.....		150	150
Chicken, gm.....		150	
Bread (toasted), gm.....		100	100
Vegetables (fresh), gm.....		100	100
Onions, gm.....		60	60
Potatoes, gm.....		60	60
Sugar, gm.....		50	50
Eggs, no.....		two (gm 60)	two (gm 60)
Rice.....			100
Butter, gm.....			30
Cream, gm.....			60
Flour, gm.....			60
Cheese, gm.....			50
Calories.....	1,084	1,916	3,001
Proteins (animal), gm.....	96.2	131.1	97.0
Proteins (vegetable), gm.....	1.8	14.0	18.0
Fats, gm.....	22.6	45.3	90.2
Carbohydrates, gm.....	91.8	215.5	295.4
Proteins/Fats/Carbohydrates ratio.....	1/0.23/0.95	1/0.31/1.48	1/0.78/2.57

* From "Gastroenterology" vol. 8, p. 732, June 1947 (108).

** The milk was served as junket. Milk was first skimmed; then rennet was added and the junket was kept in ice box overnight.

*** The liver was served as liver soup. The liver was cleaned of blood as much as possible. The pulp was then ground and sieved. Orange juice or vanilla were added for flavoring and the soup was served chilled. After a few days addition of a little sugar (10-20 gm.) was permitted.

**** or Marmite: total extract of yeast.

firmed at post mortem. It appears then that, at least in our series, definite lesions of the gastrointestinal tract were present and that their severity was related to the degree of severity of the condition, as permanent lesions became established in the few cases in which the disease appeared clinically irreversible.

5. *Prognosis.* The prognosis of the disease was favorable especially when

treatment was started early. All primary or relapsing cases, with the exception of the four chronic patients, recovered completely under therapy. As will be discussed at more length in describing the treatment of the syndrome, diarrhea, meteorism and muscular cramps were controlled almost immediately. Deficiency symptoms also disappeared rapidly and the patient showed a steady increase in weight and strength. Blood findings, fat absorption, and distribution of fecal fat, returned to normal. A large number of cases have been followed since their repatriation to Italy and none, to our knowledge, has presented any further sign of activity of the syndrome. The 23 patients who relapsed at every rainy season have enjoyed perfect health since their return to Europe. The only cases still needing treatment were those classified as chronic. This low incidence of therapeutic failures (9 cases) indicates the favorable prognosis of the condition. The observation that 8 out of 9 chronic cases are older than 45 years confirms the statement by Manson-Bahr (68) and Fairley (35) that old age represents a definite handicap to recovery. The data given also emphasize the advisability of moving cases of tropical sprue away from areas where the disease thrives, especially if relapses are to be avoided.

The more favorable prognosis in our patients than in those studied by other authors was probably due to a lesser degree of severity and the institution of treatment early. Thus, Elder (33) reported that of 400 British soldiers suffering from sprue who required repatriation from India only 12% were symptom-free at the end of the first year, 10% had suffered relapses during that period and 78% still presented acute symptoms. It should be mentioned, however, that his patients were all soldiers on active duty likely to report for treatment later.

In determining recovery we followed essentially the criteria laid down by Fairley (35): improvement of the general condition and return of the weight to normal, regression of diarrhea and of the deficiency symptoms, reversal of the abnormal blood findings and resumption of normal fat absorption and fecal fat distribution. However, we considered the maintenance of clinical recovery under an unrestricted diet the only safe criterion of therapeutic success since in 8 cases, in which treatment had brought about an apparently complete recovery with full regression of symptoms and even return to normal of fat absorption, relapses were observed when a normal diet was resumed.

6. *Treatment.* The treatment which will be outlined is far from ideal. Because of war restrictions, many items were strictly limited or unobtainable in sufficient quantities at the proper time. For example, parenteral liver extract was made available only for cases with severe macrocytic hyperchromic anemia; liver soup and total yeast extract were offered for all the other cases.

Diet and rest represented the basis of treatment in the majority of cases. Patients showing signs of activity of the disease were kept in bed as much as possible. As for the diet, after much experimenting, three types were adopted which appear in Table 11. Diet 1, high in animal proteins with a protein/fat/carbohydrate ratio (P/F/C) of 1.0/0.23/0.95, was given to patients newly admitted to the hospital with severe acute symptoms of the disease. These patients seemed to tolerate the diet well, therefore it was continued for a week or

longer. In several cases small ulcerations of the tongue required painting with a 4% cocaine hydrochloride solution to make feeding possible in the few initial days of treatment. Diet 2, which had a P/F/C ratio of 1.0/0.31/1.48, followed. It was continued until regression of active symptoms. Diet 3, with a P/F/C ratio of 1.0/0.78/2.57 was given to all convalescent patients until clinical recovery appeared complete. Notwithstanding its high content of fats and carbohydrates, this diet was found to be efficient, as judged by the weight curve of the patients.

Some of the items of the diet required special preparation. Milk in diets 1 and 2 was given as unsweetened junket several times daily in order to avoid an increase of meteorism. In diets 1 and 2 liver was given as liver soup (see Table 11 for details of its preparation). Vegemite and Marmite, which are whole extracts of yeast, were often refused by the patients with severe glossitis because of their high content of salt. Therefore, it was necessary to dilute them with water and give them as fluid in the 24-hour period. Frequent small feedings were found to be tolerated best by the patients still presenting active symptoms, for bulky meals contributed to the perpetuation of diarrhea and meteorism. Items of diet 1 were divided into seven light meals given at 2 hour intervals from 8:00 a.m. to 10:00 p.m., as shown in the following scheme. The weights of milk, fruit and liver represent the amount of original foodstuff from which milk junket, fruit juice and liver soup, respectively, were prepared.

8:00 a.m. milk (350 gm.)
 10:00 a.m. fruit (tangerines or oranges, 125 gm.), liver, (125 gm.)
 12:00 a.m. tomato juice, (50 gm.), white of a boiled egg (25 gm.)
 2:00 p.m. milk (350 gm.)
 5:00 p.m. milk (350 gm.)
 7:00 p.m. fruit (125 gm.), liver (125 gm.)
 10:00 p.m. milk (350 gm.), white of a boiled egg (25 gm.)
 plus Vegemite or Marmite (50 gm.) in water in the 24 hour period.

Items of diet 2 were also divided into 7 meals given at about 2 hour intervals. The diet was modified in some cases to agree with the particular taste of the patient. However, substitutions were carefully selected in order to maintain the original nutritive values. White sauce or mayonnaise were usually given with chicken, mutton or vegetables. In this case the necessary quantity of milk, eggs or sugar was subtracted from the total amount of these items allowed daily and small quantities of flour were added to the ration.

8:00 a.m. milk (300 gm., as junket), sugar (15 gm.), Vegemite (15 gm.) in water
 10:00 a.m. egg (boiled), tomato juice (50 gm.), chicken (150 gm., boiled), bread (50 gm., toasted), vegetables (50 gm., boiled)
 12:30 a.m. liver (90 gm., as soup), fruit (pulp of banana or mango or tangerines, 125 gm.)
 2:30 p.m. milk (300 gm., as junket), sugar (15 gm.), Vegemite (15 gm., in water)
 5:00 p.m. liver (90 gm., as soup), egg (boiled), potatoes (60 gm., boiled), onions (60 gm., boiled)
 7:00 p.m. mutton (150 gm., boiled), bread (50 gm., toasted), vegetables (60 gm., boiled), fruit (pulp of banana or mango or tangerines, 125 gm.)
 10:00 p.m. milk (300 gm., as junket), sugar (20 gm.), Vegemite (20 gm., in water)

Items of diet 3 were served as 5 meals according to the following schedule. Liver was given not only as soup (when so preferred by the patient) but also grilled or fried with butter, onions, or vegetables. A more liberal use of sauces was allowed to flavor vegetables and mutton. Flour was used in cooking the sauces or for occasional preparation of macaroni. Boiled rice was usually served with butter.

- 8:00 a.m. milk (350 gm.), sugar (20 gm.), cream (15 gm.), egg (boiled), Vegemite (15 gm., in water)
- 10:00 a.m. rice (50 gm., boiled), butter (15 gm.), tomato juice (50 gm.), liver (80 gm., grilled or fried), fruit (125 gm.)
- 12:30 a.m. milk (250 gm.), cream (15 gm.), sugar (10 gm.), Vegemite (15 gm., in water), bread (50 gm., toasted), mutton or fish (150 gm., steamed or boiled)
- 5:00 p.m. milk (250 gm.), cream (15 gm.), sugar (10 gm.), Vegemite (15 gm., in water), bread (50 gm., toasted), liver (100 gm., soup or grilled), egg (boiled), rice (50 gm., boiled), butter (15 gm.), fruit (125 gm.)
- 10:00 p.m. milk (150 gm.), cream (15 gm.), cheese (50 gm.), sugar (10 gm.)

In most of the cases in the first and second stage of the disease, when treatment consisted of rest and diet, with ample quantities of liver soup and whole yeast extract, improvement was prompt. The number of stools and meteorism decreased considerably in a period of 2 to 4 days and the feces became semisolid. Intestinal functions were restored to normal in a week; while 30 days after the beginning of treatment fat absorption and distribution of fecal fat were within normal limits (Table 12). The patients began to gain weight rapidly. Some gained from 10 to 15 lbs. a week until their original weight was reached or passed. Their appetites became ravenous. With the regression of diarrhea, meteorism and anorexia disappeared almost immediately and in a period of 2 or 3 weeks asthenia, hypotension, paresthesias, and muscular cramps abated. Thirty days after the beginning of treatment blood glucose fasting levels and oral glucose tolerance curves were found to be normal (Table 13). A normal HCl content of the stomach juice and a normal response to the Cade and Milhaud's test meal were found in five patients examined about 30 days after the beginning of treatment. On the other hand, symptoms of deficiency such as dermatitis, pigmentation, glossitis and stomatitis responded slowly to diet and rest alone. Glossitis and angular stomatitis cleared up in about 10 days when 100 mg. of nicotamide were administered daily for 10 days and 6 mg. of riboflavin daily for 15 days. Dermatitis and pigmentation often persisted long after the clinical remission of other symptoms.

Fatty diarrhea responded to diet alone. It was noticed, however, that bismuth salicylate or sulfaguanidine resulted in a rapid alleviation of the diarrhea in the first or second stage of the disease or in relapses. A minimal and inconstant effect was observed in patients with severe macrocytic anemia; no effect was achieved in chronic cases. Bismuth salicylate was used in a dose of 1 gm. t.i.d., in 268 cases. It reduced the meteorism and diarrhea almost immediately and improved the consistency of the stools in 48-72 hours. Sulfaguanidine was given to 175 cases in a dose of 4 gm. initially, 2 gm. every 3 hours in the first day,

longer. In several cases small ulcerations of the tongue required painting with a 4% cocaine hydrochloride solution to make feeding possible in the few initial days of treatment. Diet 2, which had a P/F/C ratio of 1.0/0.31/1.48, followed. It was continued until regression of active symptoms. Diet 3, with a P/F/C ratio of 1.0/0.78/2.57 was given to all convalescent patients until clinical recovery appeared complete. Notwithstanding its high content of fats and carbohydrates, this diet was found to be efficient, as judged by the weight curve of the patients.

Some of the items of the diet required special preparation. Milk in diets 1 and 2 was given as unsweetened junket several times daily in order to avoid an increase of meteorism. In diets 1 and 2 liver was given as liver soup (see Table 11 for details of its preparation). Vegemite and Marmite, which are whole extracts of yeast, were often refused by the patients with severe glossitis because of their high content of salt. Therefore, it was necessary to dilute them with water and give them as fluid in the 24-hour period. Frequent small feedings were found to be tolerated best by the patients still presenting active symptoms, for bulky meals contributed to the perpetuation of diarrhea and meteorism. Items of diet 1 were divided into seven light meals given at 2 hour intervals from 8:00 a.m. to 10:00 p.m., as shown in the following scheme. The weights of milk, fruit and liver represent the amount of original foodstuff from which milk junket, fruit juice and liver soup, respectively, were prepared.

8:00 a.m. milk (350 gm.)
 10:00 a.m. fruit (tangerines or oranges, 125 gm.), liver, (125 gm.)
 12:00 a.m. tomato juice, (50 gm.), white of a boiled egg (25 gm.)
 2:00 p.m. milk (350 gm.)
 5:00 p.m. milk (350 gm.)
 7:00 p.m. fruit (125 gm.), liver (125 gm.)
 10:00 p.m. milk (350 gm.), white of a boiled egg (25 gm.)
 plus Vegemite or Marmite (50 gm.) in water in the 24 hour period.

Items of diet 2 were also divided into 7 meals given at about 2 hour intervals. The diet was modified in some cases to agree with the particular taste of the patient. However, substitutions were carefully selected in order to maintain the original nutritive values. White sauce or mayonnaise were usually given with chicken, mutton or vegetables. In this case the necessary quantity of milk, eggs or sugar was subtracted from the total amount of these items allowed daily and small quantities of flour were added to the ration.

8:00 a.m. milk (300 gm., as junket), sugar (15 gm.), Vegemite (15 gm.) in water
 10:00 a.m. egg (boiled), tomato juice (50 gm.), chicken (150 gm., boiled), bread (50 gm., toasted), vegetables (50 gm., boiled)
 12:30 a.m. liver (90 gm., as soup), fruit (pulp of banana or mango or tangerines, 125 gm.)
 2:30 p.m. milk (300 gm., as junket), sugar (15 gm.), Vegemite (15 gm., in water)
 5:00 p.m. liver (90 gm., as soup), egg (boiled), potatoes (60 gm., boiled), onions (60 gm., boiled)
 7:00 p.m. mutton (150 gm., boiled), bread (50 gm., toasted), vegetables (60 gm., boiled), fruit (pulp of banana or mango or tangerines, 125 gm.)
 10:00 p.m. milk (300 gm., as junket), sugar (20 gm.), Vegemite (20 gm., in water)

Items of diet 3 were served as 5 meals according to the following schedule. Liver was given not only as soup (when so preferred by the patient) but also grilled or fried with butter, onions, or vegetables. A more liberal use of sauces was allowed to flavor vegetables and mutton. Flour was used in cooking the sauces or for occasional preparation of macaroni. Boiled rice was usually served with butter.

- 8:00 a.m. milk (350 gm.), sugar (20 gm.), cream (15 gm.), egg (boiled), Vegemite (15 gm., in water)
- 10:00 a.m. rice (50 gm., boiled), butter (15 gm.), tomato juice (50 gm.), liver (80 gm., grilled or fried), fruit (125 gm.)
- 12:30 a.m. milk (250 gm.), cream (15 gm.), sugar (10 gm.), Vegemite (15 gm., in water), bread (50 gm., toasted), mutton or fish (150 gm., steamed or boiled)
- 5:00 p.m. milk (250 gm.), cream (15 gm.), sugar (10 gm.), Vegemite (15 gm., in water), bread (50 gm., toasted), liver (100 gm., soup or grilled), egg (boiled), rice (50 gm., boiled), butter (15 gm.), fruit (125 gm.)
- 10:00 p.m. milk (150 gm.), cream (15 gm.), cheese (50 gm.), sugar (10 gm.)

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TABLE 12

Fecal fat distribution and fat absorption in patients with tropical sprue, 30 days after the beginning of treatment

(Previous results in the same untreated patients are shown in Table 10.)

CASE NO.	WEIGHT DRY FECES	TOTAL FAT	TOTAL FAT				ABSORP- TION	S/U RATIO	S/SF RATIO
			Split	F.A.	Soaps	Unsplit			
Patients in the first or second stage of the disease									
	gm	%	%	%	%	%	%		
19	32.5	10.1	75.3	32.6	42.7	24.7	87.4	3.05	0.57
627	29.4	10.3	74.2	34.3	39.9	25.8	87.1	2.87	0.54
903	29.7	8.9	77.4	41.7	35.7	22.6	88.7	3.42	0.46
1012	33.2	12.0	75.9	33.9	42.0	24.1	85.0	3.15	0.55
Average...	31.2	10.32	75.70	35.62	40.08	24.30	87.0	3.12	0.53

Patients in the third stage of the disease

49	28.5	12.4	79.2	40.7	38.7	20.6	84.0	3.84	0.49
524	31.6	10.2	71.5	29.4	42.4	28.2	87.2	2.54	0.59
684	39.6	9.0	75.4	37.2	38.2	24.6	88.5	3.07	0.51
792	18.4	8.7	81.0	38.8	41.5	19.7	89.2	4.11	0.51
959	16.2	11.0	77.1	36.5	40.6	22.9	86.3	3.36	0.53
1062	29.2	11.2	75.9	37.9	38.0	24.1	86.1	3.15	0.50
Average...	27.25	20.91	76.68	36.75	39.90	23.35	86.9	3.34	0.52

F. A., Fatty Acids; S/U Ratio, Split/Unsplit Fat Ratio; S/SF Ratio, Soaps/split Fat Ratio.

The values given are an average of daily determinations over a three day period.

TABLE 13

Blood sugar concentrations during oral glucose tolerance tests in 10 patients with tropical sprue, 30 days after the beginning of treatment

(Results in the same untreated patients are given in Table 8)

CASE NO.	TIME				
	0 min.	30 min.	60 min.	90 min.	120 min.
Patients in the first or second stage of the disease					
182	97.4	143.9	162.6	185.2	162.7
502	102.6	152.8	179.3	210.5	168.4
627	98.4	144.6	167.1	178.6	152.8
903	95.7	139.5	160.2	189.3	143.5
Patients in the third stage of the disease					
67	100.6	154.1	176.9	207.2	169.1
198	98.7	143.7	169.6	193.7	170.5
524	104.2	162.2	184.2	220.4	178.5
618	100.4	170.9	193.1	215.9	184.2
899	95.3	150.8	178.7	202.6	175.4
954	99.5	147.2	172.8	198.5	175.3
Average.....	99.28	150.97	174.45	200.19	168.06

... 100 ml. of blood. The patients received 1 gm.

and 1 gm. every 3 hours in the 3 following days. Its administration resulted in the arrest of the diarrhea and in an immediate feeling of well-being by the patient. The results were striking enough to recommend the routine use of the drug for the treatment of diarrhea in all patients in the initial stages, but unfortunately the limited supply interfered with such a project. The same striking response of early stages of sprue to sulfaguanidine was observed by all authors who have reported recently on the disease in India (21, 55, 118, 33). This confirms the communication made as early as 1938 by Rogers (94) on the effectiveness of prontosil in sprue. The mechanism of action of sulfaguanidine in the disease is not established and no studies of its influence on fat absorption or on its preventive activity have been carried out. A large scale experiment on the preventive effect of the administration of sulfaguanidine to all inmates in the Camp of Yol had been planned for the period June-September 1944 but it could not be carried out because of insufficient quantities of the drug. The activity of sulfaguanidine became less evident with the progression of the disease until it appeared ineffective in controlling the diarrhea of the very advanced cases. This would seem to indicate that the drug is not a specific. As Walters (118) pointed out, the action of the drug consists in suppressing the secondary infections which may be responsible, at least in part, for the severity and persistence of diarrhea.

The treatment of the 153 cases of macrocytic anemia was based on the administration of liver extract. Two different preparations were used: Lilly's "Liver extract" (obtained through the courtesy of the International Red Cross) containing 10 U.S.P. Units per cc; and the "T.C.F. Liver Extract" (sheep liver extract prepared by the Teddington Chemical Factory in Bombay) described as containing "most of the B-complex substances present in the original liver". The British Authorities supplied the "T.C.F." as well as an insufficient number of samples of "Hepatex" and "Hepastab." We were unable to determine how much liver was represented in 1 cc. of "T.C.F." extract. After a preliminary trial, Lilly's extract was given in a dose of 2 cc. daily for the first week, then every third day for the following 2 weeks. Finally, the hospitalized patients were given a weekly maintenance dose of 2 cc. They were advised to continue this in their camp but very few were able to do so. When the "T.C.F." preparation was used higher doses were found necessary; hence 4 cc. were administered daily for the first week, then 2 cc. every other day for the two following weeks, and finally a maintenance dose of 2 cc. twice a week. The injections were given intramuscularly and no reactions occurred. All patients showed a satisfactory response. A marked reticulocytosis was observed from 4 to 6 days after the beginning of the treatment; it reached a maximum of 7 to 48% after 7 to 10 days; and then gradually declined to normal values in 3 or 4 weeks. At the same time the number of red and white blood cells in leucopenic subjects increased rapidly and reached normal levels at the end of 4 weeks. Hemoglobin percentages reached normal values in about 4 weeks, after an initial limited decline. These findings are summarized in Figure 3 which shows the average changes of hemoglobin, red blood cells and reticulocytes in 100 of our cases under parenteral liver therapy. In 32 individuals the initial drop in hemoglobin

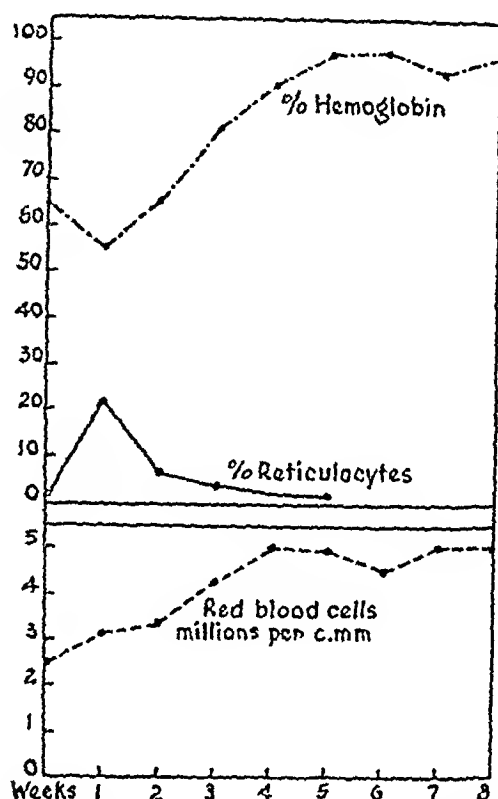


FIG. 3. HEMATOLOGIC RESPONSE TO PARENTERAL LIVER EXTRACT THERAPY IN PATIENTS WITH TROPICAL SPRUE

Average has been determined from 100 cases

TABLE 14

Sternal bone marrow in 6 cases of tropical sprue 7 days after the beginning of treatment with optimal doses of liver extract parenterally with satisfactory reticulocytic crisis

(The count was on 500 elements. For figures of the same untreated cases see Table 6.)

CASE NO.	ME.	E. ER.	L. ER.	NORM.	GR. S.	LY. S.	PLAS.	MEGAK.	NON C.C.
	%	%	%	%	%	%	%	%	%
196	1.3	3.5	11.3	26.2	51.8	4.6	2.0	0.3	0.0
524	2.4	7.0	19.8	27.0	32.9	8.7	0.8	0.8	0.6
820	0.4	1.7	10.8	28.4	51.1	5.6	0.2	0.6	1.2
907	4.7	6.0	14.7	19.2	47.2	7.8	0.0	0.0	0.4
978	1.9	4.7	15.0	20.3	46.2	9.0	0.7	1.2	1.0
1027	0.8	3.2	18.1	22.5	48.6	6.4	0.3	0.1	0.0
Average...	1.90	4.35	14.95	23.93	46.30	7.02	0.67	0.50	0.53

Me., Megaloblasts; E. Er., Early erythroblasts; L. Er., Late erythroblasts; Norm., Normoblasts; Gr. S., Granulocytic series; Ly. S., Lymphocytic series; Plas., Plasma cells; Megak., Megakariocytes; Non C.C., Non classifiable cells.

level and the concomitant increase in the number of red blood cells were so extensive as to give a temporary picture of hypochromic anemia. In these

cases, liver therapy was associated with iron in the form of ferrous sulfate (2-3 gm. daily for 1 week) or of reduced iron (0.8 to 1 gm. daily for one week) suspended in water after addition of a few drops of HCl. Our observations confirm the experience of others that in tropical sprue the response of anemia to parenteral liver therapy is usually slow, and higher doses are required than in a responsive case of pernicious anemia.

Sternal bone marrow biopsies were continued at weekly intervals during treatment in six cases. Definite changes were observed at the end of the first week. The number of normoblasts increased considerably, at the expense of the megaloblasts and erythroblasts, an observation made by Rhoads and Castle (90) (Table 14). Fifteen days after the beginning of treatment, the bone marrow was normal. No other cases were examined and no more biopsies were performed.

The response to therapy in the third stage of the disease was equally satisfactory but was somewhat slower than in the less advanced stages. Relief from diarrhea and meteorism was obtained in from 7 to 10 days. Intestinal functions appeared normal and studies of the feces revealed a normal fat distribution and fat absorption (Table 12) thirty days after the beginning of treatment. Other clinical symptoms improved with the control of the fatty diarrhea as was observed in patients treated in earlier stages. Thirty days after starting treatment the fasting blood glucose concentration, the oral glucose tolerance curve (Table 13) and the serum calcium and phosphorus concentrations (Table 15) were within normal limits. The fasting gastric acidity and the response to Cade and Milhaud's test meal were found normal in 15 cases one month after the beginning of treatment.

Seventeen of the 153 patients treated did not respond to parenteral liver therapy. This number includes the 5 who developed aplastic anemia and the 4 who became chronic cases. The 8 remaining cases, who had a severe macrocytic anemia, showed only a moderate reticulocyte response and a limited hematologic response under optimal doses of liver extract until they received transfusions (5 cases) or intramuscular injections of small quantities of blood (3 cases). Usually 2 or 3 transfusions (5 ml. of whole blood per Kg. of body weight) were sufficient to obtain a satisfactory response. As difficulty was experienced in obtaining blood in large quantities because of the small number of volunteer donors among the undernourished population of the camp, 3 more cases received intramuscular injections of small quantities of blood, 20 ml. daily for 15 to 20 days. A satisfactory reticulocyte response was obtained in patients previously resistant to parenteral liver therapy. These findings confirm the statement by Keele and Bound (55) that there are patients with advanced sprue in whom no satisfactory response to liver therapy can be obtained without a preliminary large whole blood transfusion. By what mechanism the same effect can be obtained by means of small and repeated intramuscular transfusions remains at present a matter of speculation (108).

The difficulty in obtaining blood for transfusions made it particularly difficult to treat the 5 cases of aplastic anemia. The original plan for maintaining this

patients on a minimum level between 3,000,000 and 3,500,000 red blood cells and 60 to 70% hemoglobin appeared unattainable. Transfusions were given when and in the amount possible, but the treatment was clearly insufficient, as the patients grew worse and finally succumbed to intercurrent respiratory infections.

Full scale therapy had practically no effect in the 4 chronic cases. Parenteral liver extract (Lilly's) continued for months at the fully effective dose of 2 ml. (20 Units, U.S.P.) every third day, as well as a proper diet and high doses of yeast extract and nicotamide, maintained the patients in a precarious balance only. A temporary effect upon diarrhea, edema and anemia was obtained with plasma transfusions ("Lyovac", 250 ml. per infusion).

It may be said in concluding this section that the observations presented in this paper have not added substantially to the report of Castle et al. (19). Our dietary observations confirm the fact that a high protein, low fat diet causes a diminution in steatorrhea, probably on account of the lower fat intake. With the diminution of the irritant fatty material in the intestine, a diminution in diarrhea follows. On the other hand, if no substantial change in fat absorption occurs, the use of a low fat diet only produces an apparent clinical remission and doubt remains if it has a real curative value in sprue, but merely acts as an adjuvant.

Our findings confirm the impression that liver and yeast extract are highly effective in the treatment of the sprue syndrome. They do not, however, permit an evaluation of their comparative effect as most of the patients received yeast extract and parenteral liver simultaneously. This is regrettable since discordant reports on the therapeutic activity of liver, especially when given parenterally, have been presented in the literature. It is difficult to evaluate the cause of these discrepancies, but it is certain that different preparations of liver extract vary in their therapeutic activity and generally appear to be more active in the "crude" than in the "refined" form (30). While the therapeutic activity of liver is generally accepted since the work of Castle and his group (19), it is also known that liver extract does not control diarrhea nor does it restore the intestinal function in all cases (116). Furthermore, liver alone without proper diet will not give a satisfactory therapeutic response (91) nor will it prevent relapses of the gastrointestinal symptoms (92). Possibly this variation in activity may be due to the variable content of the liver extracts in pteroyl-glutamic acid (26, 27, 111, 100) or its conjugates (60, 110), both considered specific in the treatment of sprue by most observers (28, 23).

Fat absorption studies possess considerable merit in determining the efficacy of different forms of treatment of tropical sprue. The observations reported to date are few and not in agreement with the clinical experience. Thus, high doses of yeast extract have been found to be active in restoring normal fat absorption, while liver extract, nicotamide and riboflavin appear inactive in this respect (6). Folic acid seems to be less active than total yeast extract (28). Obviously, more extensive observations based on fat absorption studies are necessary as fat absorption appears to be a reliable index of the extension,

severity, reversibility or irreversibility of the intestinal lesion. These factors will ultimately decide the success or the failure of any form of treatment in the sprue syndrome.

Behavior of the edema during treatment permitted some interesting observations. 129 patients suffering from sprue with macrocytic anemia had edema, limited to the face and ankles or generalized. When the anemia, clinical symptoms and laboratory features began to improve under liver therapy, the edema disappeared within 7 to 10 days in 69 patients. In the other 43, the occurrence of the reticulocyte response and the improvement of the hematologic conditions were accompanied by extension of the edema. Some of the patients gained 14 to 16 lbs. in a period of 3 to 4 days. Later, edema regressed with concomitant marked increase of diuresis, and finally it disappeared completely in 25 to 30

TABLE 15

Serum calcium and phosphorus concentration in patients with tropical sprue 30 days after the beginning of treatment

(All cases presented macrocytic anemia. For results in these patients before treatment see Table 7.)

CASE NO.	CALCIUM	PHOSPHORUS
	mg. per 100 ml.	mg. per 100 ml.
27	9.4	4.2
56	8.9	4.1
152	9.2	4.5
196	8.7	4.2
303	9.7	4.2
684	9.8	3.9
847	10.1	3.8
954	9.6	4.8
975	9.7	3.8
987	11.0	5.0
Average	9.61	4.25

days. Simultaneous determinations of the serum total protein level by the method of Bierry and Vivario as modified by Guillaumin, Wahl and Laurencin (41) were performed every 5 days in 10 of these cases after the beginning of liver therapy. This same study was extended to 10 cases in which edema quickly improved and disappeared under treatment. Average findings are presented in Figure 4. It was found that in the first group of cases, extension of the edema, not associated with albuminuria or hypertension, was accompanied by a greater temporary fall of total serum proteins. This was possibly due to a greater demand of plastic material for active erythropoiesis as previously suggested by the writer (108). Relationship between hypoproteinemia and extension of the edema under liver therapy was further demonstrated by the prompt regression of the edema after plasma transfusions (250-300 ml.).

These findings agree with the previous observations on the subject. The

extension of a pre-existent edema during the improvement of the clinical symptoms in the course of antianemic therapy ("delayed edema") has been observed in patients with microcytic (49) and macrocytic (29, 48) anemia, with sprue treated with pteroylglutamic acid (27) and in patients recovering from malnutrition (103, 119). In these previous observations as well as in ours (108) extension of the edema occurred from 5 to 7 days after the beginning of the treatment and was accompanied by a drop in serum protein.

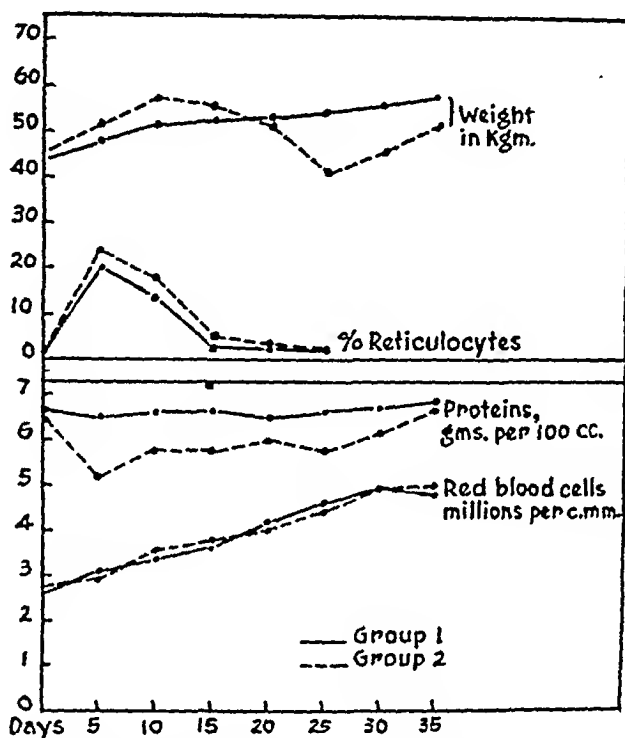


FIG. 4. RELATIONSHIP BETWEEN TOTAL SERUM PROTEIN LEVEL, EDEMA AND HEMATOLOGIC FINDINGS IN SPRUE PATIENTS DURING PARENTERAL LIVER THERAPY

The study includes 10 patients (group 1) in which edema rapidly subsided and 10 cases (group 2) in which reticulocyte crisis and hematologic improvement were accompanied by temporary extension of the edema.

DISCUSSION AND CONCLUSIONS

The conclusions are based on the epidemiologic clinical and laboratory observations presented analytically in the first part of the paper as well as on a correlation of other findings with ours from the Indian war theatre. Table 16 shows the incidence of the more common symptoms of the disease in groups of cases which have been described in sufficient detail by the several authors.

1. *Tropical Sprue in War Time India.* The review of the war time reports is not intended to be complete and includes only the observations related to our findings. Some authors have classified the syndrome described by them as "dietary deficiency" (118), "anemia with dyspepsia" (51), "nutritional macro-

cytic anemia" (2), and "parasprue" (21, 104, 105). The reasons why these different diagnoses should be changed to that of tropical sprue will be given later.

A series of 126 cases of "dietary deficiency syndrome" occurring in an Indian battalion stationed in Burma with a clinical picture similar to that seen in our patients has been presented by Walters (118). The high incidence of deficiency symptoms and macrocytic anemia may be due to the fact that statistical data

TABLE 16
Incidence of symptoms in different groups of patients with tropical sprue

AUTHOR*	CHAUDURI & CHAUDURI (1944)	KEELE & BOUND (1946)	KEELE (1946)	WALTERS (1946)	ELDER (1947)	STEFANINI (PRESENT PAPER)
NUMBER OF CASES STUDIED	22	600	80	126	400	1069
NATIONALITY	Mixed	British	British	Indian	British	Italian
Symptoms (incidence per one hundred)						
Diarrhea	100	100	90	100	100	100
Dyspepsia	90	94	91	—	—	100
Nausea	9	—	—	—	—	5.8
Vomiting	—	18	44	—	—	0.5
Loss of weight	100	100	94	78	100	100
Fever	18	—	—	—	—	12.1
Glossitis	96	96	61	—	08	90.4
Angular stomatitis	—	40**	14	—	44	27.22
Catarrhal proctitis	—	—	—	—	—	29.47
Skin changes	—	27	9	52	18	33.67
Pigmentations	—	4	—	—	—	32.70
Muscular cramps	—	25	25	—	—	27.20
Hypotension	—	8	—	—	—	92.4
Paresthesia	—	—	—	04	—	17.4
Hyperchromic anemia	50	—	26	20	22	14.4
Edema	28	—	—	—	—	10.2

The sign — means that no information on the incidence of the single symptoms is available in the original paper.

* Figures relative to the cases described by Bramwell-Cook (12) are not given for insufficient information was available in the original paper.

** Including cheilosis.

were based on studies of hospitalized patients already in an advanced stage of the disease. Walters stressed the occurrence of the syndrome among vegetarians and considered the dietary deficiency of animal proteins a definite predisposing cause. This dietary deficiency could also explain the high percentage, found in this group, of cases of considerable severity who did not respond satisfactorily to treatment.

Sixty cases of "anemia with dyspepsia" among Indian troops in Burma described by Howell (51) and five selected cases of "nutritional macrocytic anemia" in a unit of vegetarian Indian soldiers stationed in Egypt reported by Balbir

Singh (2) showed characteristics similar to those of the patients studied by Walters.

The results obtained by Keele and Bound (55) and Keele (54) in a series of 680 cases of tropical sprue occurring among British soldiers stationed in India are important because of the large number of cases studied, the careful clinical observation and the thorough investigation from a biochemical point of view (see papers by Black and others: 7, 10, 9, 8, 6, 38). The clinical picture of these cases was very similar to that seen in our series of patients who had reached the late stages of the disease. This is not surprising because the subjects studied by the British authors required hospitalization or were waiting for repatriation on medical grounds. A comparison of our findings with those obtained by Keele and Bound and Keele shows only a few significant discrepancies: Vomiting: 0.5 and 18%, hypotension: 92 and 8%, pigmentation: 33 and 4%, respectively. This contrast may be explained by differences in ethnical group, diet and habits of the patients. Interestingly, the feces of Keele and Bound's patients presented marked steatorrhea with a very high ratio of split to unsplit fecal fat (from 3:1 to 20:1). This finding may be due to some technical error and will be discussed later. Similar findings in 400 patients, cases which include many of those studied by Keele and Bound, have been reported by Elder (33).

During the period 1942-1945 interesting clinical syndromes closely related to sprue were described among the civilian population of India. Chauduri and Rai Chauduri (21) made a detailed study of 22 cases of a syndrome, commonly encountered in Calcutta among both Indians and Europeans, characterized by asthenia, dyspeptic symptoms, pale diarrhea with moderate steatorrhea, glossitis, macrocytic anemia and diagnosed as "parasprue." Closely related to these findings is the syndrome described by Bramwell-Cook (12) found among natives of the Gujerati district and diagnosed by the author as "vitamin B deficiency." Fat absorption studies were not carried out in either series, but clinical symptoms, laboratory results including a flat oral glucose tolerance curve, increased fecal fat and therapeutic response to a low fat, low carbohydrate, high protein diet and to parenteral administration of liver extract justify the diagnosis of tropical sprue.

A study of the clinical picture of each of these syndromes described reveals similar characteristics. Minor differences in the incidence of single symptoms and in the severity of the condition may be attributed to the differences in race, diet and evolution of the disease. Nor should it be forgotten that preexisting debilitation or dietary deficiency may have modified considerably the course of sprue and its response to treatment. For example, it has been observed constantly that anemia develops much earlier and therapeutic response is much slower in vegetarian Indians than in well-fed Europeans. A similar clinical picture, constant presence of steatorrhea with an increased ratio of split to unsplit fecal fat, decreased fat absorption, and a favorable response to yeast and liver extract are features common to cases described and should therefore be

diagnosed as tropical sprue. Confirmatory evidence is the evolution from a partial or mild clinical picture to a fully developed condition of tropical sprue when the disease is left untreated for a period of time.

2. *Diagnosis of Tropical Sprue and Allied Syndromes.* The diagnosis of sprue is often difficult especially in the non-endemic areas. The disease may be missed altogether or its recognition delayed until it presents the complete clinical picture. At this stage, less reversible lesions have developed and resistance to treatment and the possibility of relapses are much higher. Milder forms, initial and early stages of the disease which appear frequently are vaguely classified as atypical or incomplete and are called "nutritional diarrhea", "hill diarrhea", "parasprue", to quote only a few of the terms. The author himself erroneously used the clinical designation of "parasprue" in a preliminary report published in 1945 (104), but he believes now that terms of this type are misleading if not meaningless and that the differentiation of these syndromes from tropical sprue is often artificial and confusing. Illustrative of this fact is the line of thought of Chauduri and Rai Chauduri (21) who, after having attempted to distinguish "parasprue" from tropical sprue on the basis of the different racial distribution, type of diarrhea, "absence of very severe emaciation and peculiar waxy colour," and improvement on parenteral liver therapy only without any strict dietary regime, conclude that "the condition appears to be the Indian version of sprue." Obviously, the lack of accepted diagnostic criteria is the main obstacle in correctly recognizing tropical sprue. Little can be expected from the multiplication of clinical terms which cannot possibly cover the protean manifestations of the "sprue syndrome." We agree with Stannus (102) that a definition or classification of sprue is hardly possible or conceivable until more is known of its etiology, pathogenesis and significance.

The modern concept of the diagnosis of tropical sprue is based upon clinical criteria and upon a few laboratory findings which may include steatorrhea, very often determined with microscopic examination of the feces without proper chemical control, a flat oral glucose tolerance curve, and hypocalcemia. These findings are presumed to show the presence and the extension of the intestinal dysfunction in sprue. The limitations of these diagnostic methods are obvious and have been analyzed by Stannus (102). The discussion which follows will not offer any new or better method of diagnosis but it will attempt to prove that when these methods are at hand they can be better used for a correct understanding of the fundamental nature of the disease.

It is agreed that the basic defect in sprue is represented by a partial breakdown of the digestive functions. Partial, because digestion and to a certain extent transportation of food, take place normally while only absorption appears to be invariably impaired. The hypochlorhydria sometimes found in sprue is never of such a degree as to interfere, theoretically at least, with the activity of pepsin or of the pancreatic and intestinal enzymes as determined by direct and indirect methods. Recently however, a disturbance in the intestinal digestion of mucus in sprue has been demonstrated (98) by means of intestinal intubation.

This finding may partly explain the defect of emulsification of fats and fatty acids. According to the views of Frazer (39) this defect of emulsification may be an important factor in the pathogenesis of malabsorption of fats.

Intestinal motility and transportation of chyle in the intestine of patients with tropical sprue have been analyzed mainly by radiologic techniques. Mackie, Miller and Rhoads (67) demonstrated a derangement of the normal motor function and abnormal variations in the tone of the muscular layer of the intestinal wall. Furthermore, Golden (40) has described a radiologic picture that he considers specific for the "sprue syndrome" and avitaminosis B, consisting in abnormalities of the motility of the villi and flattening of the valvulae conniventes ("deficiency mucosal pattern"). For some authors this condition would represent the main factor of the deficient intestinal absorption found in the "sprue syndrome." According to Hurst (52) a vitamin deficiency or toxemia would cause a dysfunction of Meissner's plexus and subsequently paralysis of the muscularis mucosae which would in turn cause the loss of pumping action of the villi. This theory gives paramount consideration to the malabsorption of fats, which is not the only defect in sprue. Many authors have refuted this view, because it is not supported by any clinical or experimental fact showing that the defect of motility of the villi is the primary factor interfering with the normal absorption of food. On the contrary, it is known that villi can be quite motionless during absorption (120) and, as Stannus (102) states: "that the pumping action of the villi normally propels the content of the lacteal radicles into the bigger lacteals cannot be doubted, but this is one matter; absorption by the epithelium lining the gut is another." The possibility of increasing dietary fat without reducing the percentage of fat absorbed (6) is another argument against Hurst's theory as are the recent findings on the mechanism of fat absorption by Frazer and the co-workers (39, 24). Finally it must be stated that theories of this type, which Stannus (102) has criticized, have given little thought to the repeated observation that the reaction of the intestinal wall to the bulky and irritant content may be responsible for the X-ray findings as much as a primary functional derangement of intestinal motility (86, 89, 53). We do not, of course, deny completely the existence of important motor abnormalities in the intestine of patients with tropical sprue for irregularities of intestinal emptying are easy to demonstrate. Ingelfinger and Moss (59) have shown with ballistographic studies that the small intestine is less resistant to distention and that its normal reaction is restored by treatment with acetyl- β methyl-choline chloride. They suggest, therefore, that in sprue the intestinal intramural nervous apparatus fails to liberate acetylcholine. In conclusion, if disturbances of motility exist in tropical sprue and allied disorders, they are concomitant and aggravating but they are probably not the primary factor in the pathogenesis of intestinal malabsorption.

Because of the length of this paper, the mechanisms of intestinal malabsorption in the "sprue syndrome" will not be discussed further. The subject in regard to fats has been revised recently by Frazer (39). A few considerations on the specificity and extension of the defect of intestinal absorption, however,

are necessary for a better understanding of the following discussion. The defect of absorption in sprue is general, since it involves all dietary constituents. This is evident from the chemical analysis of the blood and the feces which have revealed steatorrhea with a high ratio of split to unsplit fat. Biochemical findings include a low blood glucose level, a flat oral glucose and fructose (38) tolerance curve, low blood lipids after ingestion of fat (30), a low serum lipid level (62), a low plasma vitamin A and carotene level (20), a low oral vitamin A tolerance curve (58), and a low oral glycine tolerance curve (34). Moreover, the Respiratory Quotient shows only minimal and delayed changes after the ingestion of glucose (44); while rhamnose given by mouth is slowly and incompletely eliminated in the urine (106). The defect of absorption is evidently a considerable one, but the malabsorption of fats has received greater attention because accurate methods for the determination of fecal fats are available. Moreover, because of the normally slow absorption of fats, its defect in pathological conditions is more easily and more promptly recognized. When a shorter (73) or longer (113) portion of the intestine is removed surgically in man, symptoms similar to those of sprue eventually become evident. Subsequently, absorption studies reveal that carbohydrates and proteins continue to be absorbed but that fats are scarcely absorbed (113).

The defect of intestinal absorption in sprue, while severe, is nevertheless only partial. According to our results and those of Black (6, 9, 8) the absorption of fats is only 15-40% lower than in normals and steatorrhea regresses when the intake of alimentary fat is reduced, hence, apparent curative effect of a low fat diet. On the other hand, the defect of absorption is specific. As an example, the absorption of glycine is reduced in tropical sprue (34) but not in other similar conditions such as diarrhea of pernicious anemia. It should be pointed out that observations on the rate and extent of absorption of amino acids and carbohydrates in sprue and allied conditions would reveal important information on the physiological mechanism of intestinal absorption, as well as be helpful in differential diagnosis. Finally, as noted above, the wide occurrence of deficiency symptoms shows that the defect of absorption does not spare any dietary and nutritive factor. The different length of time required for their appearance is probably related to the severity of the disease, the capacity of the body to store essential factors and the requirement by the body for same.

The primary defect of intestinal absorption, which is present from the onset, becomes the essential element in the diagnosis and prognosis of the sprue syndrome because onset, development, clinical picture, course of the disease, outcome and response to treatment are all closely related to the extension and duration of the intestinal dysfunction. Study of fat absorption, as was already explained, is particularly useful in establishing the extension and severity of the impairment of intestinal absorption. Steatorrhea with a high ratio of split to unsplit fat is a good criterion for the diagnosis of syndromes of the sprue type. But the techniques employed for these studies are frequently unsuitable. Observation of the macroscopic and microscopic appearance of the stool is not a reliable method for the determination of steatorrhea. Macroscopic appear-

ance of a stool of a patient with sprue is not diagnostic, particularly in the initial stage, as it may vary in weight, consistency and color. In fact, the color may even become darker upon standing. Furthermore, none of these characteristics bears a relationship to the fat content of the stool. Even microscopic examination which reveals the absence of fatty acids, crystals or soaps is insufficient evidence to assume that the stool is not a fatty one.

Chemical analysis, when performed with satisfactory techniques, is the only reliable method for the determination of the fat content of the feces. However, in order to prevent mistakes and false interpretations of these determinations, there are two factors of importance to consider: 1) the diet and 2) the fat intake of the patient. The subject must be maintained on a diet of constant quantitative and qualitative intake of fats, proteins and carbohydrates if the results are to be considered a reliable index of the absorption properties of the gastrointestinal tract. Without this precaution, chemical analysis of the fat content of the feces usually gives variable results. Secondly, the fat intake of the patient under investigation must be close to that of a normal diet. The defect of intestinal absorption in sprue is only partial and if the patient receives a low fat diet, his steatorrhea may disappear and the estimation of the percentage of fat absorption may be grossly incorrect. Leishman (63) reports that 20% of his cases of sprue were without steatorrhea. Such reports may be due to the disregard of this precaution. Moreover, when the fat content is determined in fractions and not on the total amount of feces for the 24 hours or for the total period of observation, all components of the diet and even the amount of water given to the patient must be controlled. In this instance the amount of residual food in the feces (high or low) and the state of hydration of the stool influence the dry weight independently of the fat content.

These precautions, however, will not prevent error if the test is not run over a period of days. The results of a 24-hour determination may be altered by variations in intensity of steatorrhea which occur daily in patients with sprue as well as in normal subjects. Changes in the rhythm of the intestinal emptying such as a severe diarrhea, which greatly accelerates the intestinal transit, interfere with the normal splitting of neutral fat, cause its increase in the feces, and a decrease in the ratio of split to unsplit fecal fat. On the other hand, a slower emptying gives more time for absorption of fatty acids. Another factor that may be responsible for error is the variation of the lipolytic activity of the feces present in normals as well as in sprue patients (10). Such possibilities explain variations in results obtained by authors studying steatorrhea in a 24-hour period or in a single determination. Fairley (36) finds an almost normal ratio of split to unsplit fecal fat, while Keele and Bound (55) report a ratio as high as 20:1.

The technique of studying fat absorption over a period of days under standardized conditions with a fixed fat intake was applied first by Bassett et al. (4) to studies of the sprue syndrome or idiopathic steatorrhea. A similar method has been applied by Cooke et al. (24) in investigating the fat balance in patients with idiopathic steatorrhea. The 12 day observations of Black and his group

(6, 10) and our 3 day observations are isolated studies of patients with tropical sprue. However, the results correspond closely. Clinical experience has convinced Black et al., as well as us, of the greater reliability of the fat balance studies than of chylomicron counts or serum lipid curves as a diagnostic sign of intestinal malabsorption of fats. We are convinced that the administration of a constant amount of dietary fat and the determination of the fat balance over a period of days, determined by the use of markers, on the total amount of feces excreted during that period, minimizes the errors due to variability in rhythm of intestinal emptying, lipolytic activity of the feces and variations in residual content of food in the stools. Observations have revealed that steatorrhea, with a ratio of split to unsplit fecal fat higher than normal, and a deficient percentage of fat absorption are practically constant, are present from the onset of the condition and are, therefore, significant diagnostic findings in tropical sprue. As noted above, the impairment of fat absorption is partial and in our cases we have found an average of 67% as compared to a normal figure of approximately 88%. Black and others (10) have presented similar results and have shown that this deficiency of fat absorption is relatively constant even when fat intake is moderately increased. Another interesting finding is that while sprue and normal stools contain approximately the same amount of unsaponifiable fat, the saponifiable fraction is increased and to a large extent accounts for the higher content of solid fat in the stool of sprue patients. A high proportion of fat is present in the form of soaps, mainly of calcium, even when the feces appear to have a moderately acid reaction.

The ratio of soaps to split fecal fat is, therefore, an important diagnostic figure and was found, in our experience, in constant relationship to the gravity of the clinical picture. Despite the fact that this finding appeared to be rather constant in the course of our observations, more investigation is required before it can be accepted without reserve. It is particularly interesting that a completely opposite ratio of soaps to split fecal fat can be calculated from the tables presented by Suarez et al. (11) in a study of cases of tropical sprue in Puerto Rico. The reason for this discrepancy is at present only a matter of speculation. It may be pointed out, however, that the soap content of the stool is obviously influenced by the rhythm of intestinal emptying and the content of calcium in the diet.

3. *Clinical Picture of Tropical Sprue and Allied Syndromes.* While the incidence and type of the individual symptoms observed do not require special comment, there are a few points which merit mention. The sudden appearance of the initial symptoms is rather uncommon in sprue and will be commented upon later when discussing the epidemic occurrence of the disease. The absence of symptoms attributable to a deficiency of the liposoluble group of vitamins is also noteworthy. Elder (33), however, has attributed the skin changes or exfoliative dermatitis, which habitually persist long after all other deficiency symptoms have completely cleared up, to a vitamin A deficiency on the basis of the therapeutic response to the vitamin. This interpretation still remains doubtful because dark adaptation tests always gave normal results in patients presenting

typical skin changes. The pathogenic interpretation of other clinical symptoms did not offer any special difficulty. Thus, glossitis responded to nicotamide and angular stomatitis to riboflavin. Because these substances do not exercise any effect on intestinal absorption they may be specifics. Hypochlorhydria and deficient intestinal absorption of iron appear responsible, as pointed out by Wilder (122), for the microcytic normochromic anemia. With Vedder (115) we consider this type of anemia constant and typical of the early stages of the sprue syndrome.

An interesting feature of our group of cases is the high incidence of hypotension. No attempt had been made to correlate this finding with experimental observations. According to Black (6) hypotension, especially in the early stages of sprue, should be attributed to an abnormal loss of sodium and chloride in the watery stools. We, unfortunately, did not have an opportunity to observe the seriously ill patients with hypotension, asthenia, dehydration and peripheral circulatory failure which Black has described. Since the serum potassium level is normal (87) or diminished (45) in the sprue syndrome and the conservation of sodium and other bases by the kidneys is adequate, these findings contradict the theory, attributing the hypotension to adrenocortical insufficiency (112), and relate it to the common basis of all symptoms of sprue, the intestinal malabsorption. In support of this view it may also be mentioned that the hypotension of sprue does not respond to desoxycorticosterone.

Some comment is necessary concerning the classification of sprue by stages. Systematizing the disease in clinical types, which would certainly be useful for diagnostic purposes, is difficult because of the variable occurrence and association of symptoms in the individual cases. On the other hand, it has been shown that the impairment of intestinal absorption is fundamental to the disease and that the severity and extension are directly related to the type and progression. For this reason the course of the disease is divided into three stages. In the first stage the patient has symptoms of early dysfunction of intestinal absorption with steatorrhea and dyspepsia; in the second stage the signs of secondary deficiencies overshadow the symptoms of the initial period, and finally in the third stage the onset of macrocytic anemia, a late sign of intestinal malabsorption, is observed. This division into stages has a practical prognostic significance because the first and second stages are easily amenable to treatment. However, the third stage is often resistant and liable to relapses. It also abolishes the pseudoterms. Keele (54) agrees with this view when he writes: "symptoms at onset consist of anorexia, diarrhoea, vomiting, weakness and loss of weight. Diarrhoea is usually, but not always, first; the other symptoms follow quickly forming a clear symptom-complex which persists for a few weeks before further symptoms develop." He also considers the symptoms of the second period (glossitis, cheilosis, . . . , scaling of the skin) as signs of remission, since in his experience their occurrence coincides with a spontaneous improvement of the diarrhea and of other initial symptoms. With this latter point we cannot agree for we seldom noticed any improvement of clinical signs or of absorption of fats in the second stage of the disease. Symptoms of the first stage are modified with the progression of sprue as have been described already but they do not appear to improve to any great extent.

4. *Etiologic and Pathogenic Considerations.* A number of theories have been proposed to explain the etiology and pathogenesis of tropical sprue. Few of them, however, have stood up against the substantial increase of knowledge of the sprue syndrome, particularly of tropical sprue, gained in the last few years. For a comprehensive review the reader is referred to the chapter in Manson-Bahr's "Dysenteric Disorders," to the review by Stannus (102), and to Castle, Rhoads et al. (19).

The theory that considers sprue a deficiency disease appears to be particularly well grounded and supported by several experimental and clinical facts. It is true that in India well-to-do Europeans contract the disease as readily and frequently as starved Indians (36, 21), but in most cases of sprue one can usually obtain a history of prolonged dietary deficiency, especially that of animal protein. This observation has been repeatedly made in Puerto Rico (109, 94) and has been recently confirmed among Indian troops in Burma (118) and Egypt (2) where a greater incidence of sprue was noticed among vegetarians than in meat-eaters. The apparent hereditary (101) and familial tendencies (65) of the disease are probably also directly related to dietary factors. The therapeutic effect of a high animal protein diet, as well as the specific response to liver and yeast extract and especially to pteroylglutamic acid and its conjugates, also suggest a deficiency of some specific dietary factor. Finally, the disease has been reproduced experimentally by means of deficient diets. Clinical features of steatorrhea with hyperchromic anemia have been observed in monkeys by feeding them, over long periods of time, the diet of the poorer classes in India where the incidence of sprue is high (66, 123, 88). Day and co-workers (61) have produced a clinical picture resembling human sprue in monkeys fed a diet deficient in pteroylglutamic acid.

Of particular interest have been the observations by Radhakrishna Rao (88) who, after producing a sprue-like syndrome in monkeys fed a diet grossly deficient in proteins and vitamins, has shown that prolonged malnutrition finally led to atrophy of the mucosa of the gastrointestinal tract and irreversible impairment of intestinal absorption. These experimental findings have been recently confirmed indirectly in that malnutrition in prisoners of war in the Far East was accompanied by steatorrhea and impaired fat absorption typical of sprue (119) and that diarrhea with various deficiency symptoms usually found in the sprue syndrome were often present among the undernourished inmates of the concentration camps in Belsen (22, 64, 114), Landsdorf (32), Auschwitz (1) and Easton Holland (14). Rao's findings are also interesting from another point of view, he was able to confirm MacCarrison's observation that a protein deficient diet will be accompanied eventually by cystic degeneration of the thyroid gland. These findings may possibly explain the high incidence of non-functioning parenchymatous goitre that we observed. Over a three year period we found a higher incidence of goitre among sprue patients than in the other prisoners (incidence per thousand: 2.24 and 0.74, respectively). It may be interesting to point out that parenchymatous goitre is known to develop during the course of idiopathic steatorrhea (96, 112).

Our observations confirm the importance of dietary deficiencies in the pathogenesis of sprue. Among the British and Indian garrisons and the Italian prison-

ers of war stationed in Yol, the incidence of sprue, as shown in Table 17, was indirectly proportional to the adequacy of the diet. Fewer cases were observed among British soldiers receiving a diet high in animal proteins and fats than among Italian prisoners of war and Indian soldiers. The diet supplied to the Italians, even if sufficient in calories, vitamins and minerals, was low in fats and proteins, especially animal proteins and it was unbalanced due to an excess of carbohydrates. The monotony and sometimes unpalatability of the diet and the frequent substitution of some items of the diet with other foods of less nutritive value (for example, soya links—a sausage prepared with soya beans, flour, and a little pork meat) also contributed to the inadequacy. Likewise, the rations supplied to the Indian garrison were deficient in animal proteins on account of religious beliefs. The racial factor may be excluded because of the low incidence of the disease among Italians either employed as camp cooks or as volunteer workers, who received the same rations as the British troops on active duty. The importance of diet deficiency was further demonstrated by the critical increase in the number of the cases of sprue among Italian prisoners which occurred when, after June 1944, the diet was curtailed especially in fats (Table 18).

Other observations have shown, however, that dietary deficiency is not the only factor in the pathogenesis of sprue or that, at least, it does not explain all the peculiarities of the incidence of the disease. In India, sprue was endemo-epidemic in incidence. While cases appeared all year around, in certain areas the syndrome showed an epidemic seasonal incidence. For example, the cases reported by Keele and Bound occurred mostly in the months of May and June and ours immediately after or during the rainy season. When seasonal, climatic and local conditions and, of course, latent malnutrition, were favorable to the development of sprue, the disease struck in an almost epidemic form with a high incidence among the military units. About 10% of the total strength of Italian prisoners of war in Yol suffered from sprue in the three year period. Keele and Bound (55) state that among 8,846 medical cases evacuated from India on medical grounds, in the period 1943–1945, 1,073 suffered from sprue. Walters (118) reports that almost half of the battalion in which he carried out his observations presented symptoms of the disease. The epidemic incidence of sprue has also been stressed by Leishman (63) on the basis of his experience in RAF units stationed in Chittagong. Local factors were obviously important in the development of the syndrome. It was often observed that with diet and camping accommodations remaining the same the disease occurred in single military units when they were transferred to certain areas of India, Assam or Burma. Keele and Bound (55) state that Assam, Bengal and Bihar presented an environment favorable to the onset of sprue and it is of interest that most of their cases came from these areas. Leishman's experience is better quoted in his own words (63): "In Chittagong . . . Nine separate units in the area were affected, and in some instances as many as 50% developed the disease. In one RAF unit, within three weeks of its arrival in the Chittagong area, 10% of its personnel was down with diarrhoea which rapidly developed into the full sprue syndrome. This epidemic . . . ultimately involved several hundred cases."

Our observations also emphasize the importance of local factors Italian prisoners of war in India were stationed in 4 main concentration camps, but the dis-

TABLE 17

Incidence per thousand of tropical sprue among troops stationed in Yol

	1942	1943	1944
Italian prisoners	13 60	21 50	59 70
Indian troops*	12 05	39 30	24 15
British troops	0	0	6 00

* Hospitalized patients only

TABLE 18

Composition of the diet issued to garrison troops and Italian prisoners of war in India

		F/F/C RATIO
A) Italian prisoners of war		
Before June 1944		
protein (animal)	26 10	1 0
protein (vegetable)	51 25	
fats	68 65*	0 88
carbohydrates	535 60	6 92
calories	3 100†	
After June 1944		
protein (animal)	28 50	1 0
protein (vegetable)	71 10	
fats	42 93*	0 43
carbohydrates	452 65	4 53
calories	2,610†	
B) British troops		
protein (animal)	96 20	1 0
protein (vegetable)	33 20	
fats	144 84*	1 12
carbohydrates	442 15	3 41
calories	3,690†	
C) Indian troops		
protein (animal)	14 4	1 0
protein (vegetable)	51 25	
fats	51 31	0 84
carbohydrates	607 22	6 35
calories	3,710†	

Values for foods are in grams

* Including margarine, 21 30

† Approximately

case was observed only in the Camp of Yol The men in all the camps were living under comparable conditions of diet, but Yol differed from the others in

altitude as well as length and severity of the rainy season. Cases of sprue have been known to occur among Gurkha troops, apparently immune to the disease when living in their native mountains, shortly after they were sent into Burma. Their diet had not changed substantially nor had their camping conditions (46).

Sprue was manifest in military units wherever and whenever the favorable environmental conditions existed irrespective of dietary deficiencies, and it did not spare the natives, thought to be resistant or immune to the disease. In an area where the favorable conditions existed, the incidence of sprue among Europeans and natives was similar, especially if the natives were living out of their own district. Walters (118) found a large number of patients in an Indian battalion; Keele and Bound (55) counted many cases among the Chindits units fighting in Burma and Assam. We ourselves saw a considerable number of cases of "pale diarrhea and macrocytic anemia," an euphemism for advanced sprue, among Indian soldiers in Yol. Differences in the clinical picture such as frequency of secondary deficiency symptoms and of macrocytic anemia were possibly due to the undernourished condition of the Indian patients. The same may be said of the occurrence of sprue among the civilian population of India, often missed because of the diagnosis of chronic dysentery or chronic diarrhea of unrecognized nature. Chauduri and Rai Chauduri (21) and Bramwell-Cook (12) have described a large number of cases of sprue in the civilian population of Calcutta and the Gujerati district, respectively. We have already mentioned the high incidence of hill diarrhea found by Megaw and Gupta (75) in Kangra Valley. The statement that Indians are immune or resistant to sprue appears, therefore, to be based on inaccurate observations.

The high occurrence of sprue in particular areas could be related to the incidence of diseases favoring or predisposing to the illness in these areas, such as bacillary dysentery or malaria. The geographic distribution of bacillary dysentery and malaria in India, however, is not similar to that of sprue and the direct pathogenic importance of the two conditions is denied rather than supported by the war experience. Walters (118) found a history of "bowel infections" in a large percentage (67) of his selected group of 42 Jats and Rajputana Musulmans suffering from sprue; however, there was an equal incidence in a control group. Leishman (63) reported a previous history of dysentery in only 9% of his cases and Keele and Bound (55) in 24% of their 600 cases of sprue and in 21% of a control group. They also observed that the season of maximum incidence of sprue preceded that of bacillary dysentery in Southwestern India. We have not found this reversed seasonal incidence. As was anticipated, our figures are even lower because our soldiers were no longer exposed to the danger of intestinal infections associated with active duty in the field. We agree with Keele and Bound (55) that previous intestinal infections and disorders are of no direct importance in the pathogenesis of sprue but only represent a predisposing and possibly aggravating factor. The same is true of malaria, which certainly predisposes and exacerbates sprue, but is unlikely to be directly responsible for the development of the disease.

It may be concluded, therefore, that dietary deficiencies are largely responsible for the occurrence of the sprue syndrome, but it may also be pointed out that the

recent observations in India have stressed the importance of climatic and local factors. These conclusions are based on observations carried out under almost ideal experimental conditions, i.e., in military units leading a routine life and subsisting on a standard diet.

Other factors besides a dietary deficiency obviously play a role in pathogenesis since diet alone cannot explain the seasonal and local incidence. The occurrence of fever in some of the patients in the initial stage of sprue and the prompt response to sulfaguanidine are not necessarily signs that the disease is caused by an infection or by an increase in violence of a pre-existing intestinal infection. Fever probably expresses the effect of secondary bacterial invasions into a less resistant intestinal mucosa.

Since most of these studies were handicapped by the lack of investigative facilities under war time conditions, much fundamental knowledge of sprue has not been acquired. Leishman (63) has expressed the sentiment of those who worked on sprue in the Indian war theatre when he wrote: "Throughout this time my colleagues and I have been exasperated by our inability to add materially to the knowledge of the true nature of the disease." Pessimism is not justified, however, as these studies have contributed, even if in limited measure, to a better understanding of several clinical and etiologic aspects of tropical sprue.

SUMMARY

The epidemiologic characteristics, clinical picture and laboratory features of 1069 cases of tropical sprue observed during a three year period among Italian prisoners of war in a concentration camp in India are described. Data concerning incidence of the disease in relation to atmospheric conditions, changes of diet and age group, occurrence of relapses, frequency of the different symptoms and their possible pathogenesis, prognosis and treatment are given. The findings are correlated with those obtained by other authors who have described cases of tropical sprue in the Indian war theatre. The value of these observations is based mainly on the consideration that they have been carried out on a large group of military personnel living under uniform or at least comparable conditions of diet and climate.

The following conclusions have been reached:

- 1) The fundamental symptom of the "sprue syndrome" is the partial defect of intestinal absorption, whose etiology and pathogenesis are at the present time obscure. The onset, clinical picture, course and ultimate outcome of the disease are related closely to the extension, duration and severity of the intestinal dysfunction, which is usually moderate, reversible and amenable to treatment in mild or early cases of tropical sprue but which becomes more serious with the progression of the disease. The presence of a partial defect in intestinal absorption, especially that of fats, is therefore, a necessary element in the diagnosis of the "sprue syndrome." The use of the "fat balance technique" described in this paper makes its recognition and study reliable and easy. A series of studies in clear cut cases of sprue has revealed that every patient shows: 1) steatorrhea with the ratio of the split to the unsplit fat higher than normal, apparently due to an

increase in the saponifiable fraction of fecal fat; 2) a defect in the intestinal absorption of fats and 3) a ratio of the soaps to the split fecal fat ratio higher than usual.

2) Dietary deficiency is known to be an important factor in the development of tropical sprue. It, however, cannot be considered the only cause of the disease, inasmuch as some peculiarities of the cases described in India during the war, like the epidemic occurrence of sprue in a particular season of the year or its sudden, high incidence among military units shortly after their transfer to certain areas, cannot be explained on the basis of "dietary deficiency" alone. Thus, sprue occurred whenever and wherever favorable seasonal and climatic conditions existed, particularly among individuals with previous dietary deficiencies. Local, climatic and seasonal factors are therefore important in the pathogenesis of tropical sprue.

3) A classification of the "sprue syndrome" according to clinical types appears to be useful but difficult to establish because of the variability in the association of the symptoms of the disease. We believe a classification according to the stages of severity or extension of the intestinal dysfunction gives a better understanding of the variable clinical picture of the disease. The course of tropical sprue can be divided into three stages; first, one in which the initial symptoms of impaired intestinal absorption predominate, i.e., diarrhea with steatorrhea, dyspepsia; a second in which there appear multiple secondary deficiency signs, i.e., glossitis, stomatitis, proctitis, etc.; and a third characterized by macrocytic hyperchromic anemia. This classification would abolish the use of clinical definitions such as "parasprue," "hill diarrhea," "nutritional diarrhea," etc., in that these merely indicate either mild or initial stages of the same disease.

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Addendum

Only after the submission of this manuscript we had the possibility of consulting a recent article by Ayrey (Ayrey, F., *Trans Roy. Soc. Trop. Med. & Hyg.*, **41**, 377, 1947) who describes three outbreaks of sprue of epidemic proportions among British and Indians troops in Burma. He considers malnutrition, and in particular a prolonged dietary inadequacy of riboflavin and nicotinic acid, as the primary causal factor of the condition.

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DIABETIC GLOMERULOSCLEROSIS

CLINICAL AND PATHOLOGIC OBSERVATIONS WITH SPECIAL REFERENCE TO DOUBLY REFRACTILE FATTY CELLS AND CASTS IN THE URINE*

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In 1936, Kimmelstiel and Wilson (29), in a study of eight patients who came to autopsy, described a characteristic renal glomerular lesion, which they called intercapillary glomerulosclerosis. This was found in association with a previous history of diabetes, severe and widespread edema of the nephrotic type, gross albuminuria, frequently with hypertension and renal decompensation. In addition to the "striking hyaline thickening of the intercapillary connective tissue of the glomerulus", they described a deposition of fat with focal areas of doubly refracting lipid in the tubules and in the interstitial tissues of the kidney. It was the contention of these authors that this lesion was essentially degenerative in nature. They suggested that arteriosclerosis and diabetes may play a part in its causation.

A CHANGING CONCEPT OF THE SIGNIFICANCE OF THE GLOMERULAR LESION

Since this original description, a number of excellent reports have appeared in the literature expressing varied opinions concerning the relationship of the histologic lesion in the glomerulus to diabetes mellitus.

Originally, the renal lesion was said to be of infrequent occurrence, but later reports indicated that intercapillary glomerulosclerosis is not uncommon in diabetes. The question of the specificity of the lesion was considered by a number of workers. Siegal and Allen (45) found that in a series of one hundred consecutive non-diabetic hypertensives, this lesion occurred but once. Herbut (24) emphasized its specificity and stated that it was a reliable criterion in the autopsy diagnosis of diabetes. Glomerular lesions of this type have been described by Horn and Smetana (25) in many non-diabetic individuals with various renal diseases. They noted intercapillary glomerulosclerosis in patients with glomerulonephritis, generalized arteriolar sclerosis, and arteriolar nephrosclerosis, but were forced to the conclusion that extensive degrees of intercapillary glomerulosclerosis were always associated with diabetes. Laipply, Eitzen and Dutra (30) have found the specific lesion in 63.7% of 124 patients with diabetes, but only 11% had a severe degree of involvement. They also pointed out that some degree of intercapillary glomerulosclerosis may be found in patients without diabetes, but in association with arterial and arteriolar nephrosclerosis, chronic pyelonephritis, and in subacute and chronic glomerulonephritis. Bell (7) de-

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scribed two types of glomerular lesions characteristic of diabetes, the nodular and diffuse. The nodular lesion corresponds with the type described by Kimmelstiel and Wilson (29). Bell (7) concluded that the diffuse form of "intercapillary" sclerosis, although noted in diabetes, is much more frequent in glomerulonephritis. The spherical hyaline lesions were found only in cases of diabetes.

The clinical syndrome has undergone modification since its original description. Anson (4) found that the typical nephrotic syndrome was absent in 4 of 6 patients. He concluded that nephrosis does not seem to be an essential clinical feature. Newburger and Peters (40), on reviewing 4 of their own cases, together with the published case reports of Kimmelstiel and Wilson (29), and Anson (4), found that mild diabetes, hypertension, albuminuria and retinal vascular changes were the most prominent manifestations. They stated that although nephrotic edema was a common finding, edema associated with cardiac failure was present in 25% of their cases. This was in general agreement with Porter and Walker's (43) findings. Siegel and Allen (45) found a good correlation of the pathologic lesion with the nephrotic syndrome, but this depended chiefly on the severity of the glomerular lesion. They felt that the specific renal lesion should be diagnosed clinically when the syndrome of hypertension, azotemia, edema and hypoproteinemia and hypercholesterolemia occurs in the diabetic. They pointed out, however, that a number of their patients with the specific glomerular lesion revealed no evidence of renal damage or hypertension during life. Weiss (50) stated that intercapillary glomerulosclerosis assumed clinical interest only if the lesions were prominent and diffuse, and only then were they to be associated with albuminuria, hypoproteinemia and a tendency to anasarca.

Goodof (20) stated that the clinical diagnosis of this syndrome was justified under these circumstances—in a middle-aged patient with mild or moderately severe diabetes, having the disease for six years or more, excreting moderate to large amounts of albumin in the urine and without evidence of other renal disease.

Lefebvre and Dechard (31) analyzed seven patients with the specific lesion in the glomeruli. In this group, edema was not prominent, occurring in only two of their patients, and the cause of the edema was cardiac failure. They stated that proteinuria, arteriosclerosis, hypertension, hypoproteinemia in middle-aged individuals with mild diabetes, may be associated with intercapillary glomerulosclerosis, but such features have also occurred in persons who fail to show these

Millard and Root (37) reported that 14 of their 15 patients with the renal lesion proven at autopsy had hypertension and albuminuria. Only 3 however developed a nephrotic phase with hypoproteinemia and edema.

Rosenbusch (44) reported that glomerulosclerosis occurred as a late complication among 80 patients whose diabetes began in childhood. In the early stages, these patients manifested albuminuria with benign nephrosis, and later malignant hypertension and retinitis and cataracts. However, many of them at post mortem examination, revealed more than one form of kidney disease.

Dolger (15) recently stated that he believed that this syndrome existed with varying degrees of severity in every instance of diabetes mellitus of some duration. However, we were unable from his paper to determine the extent of post mortem confirmation. Retinopathy usually presaged vascular degeneration. 50% of his patients had hypertension and albuminuria at the time of the earliest retinal hemorrhage.

Laipply, Eitzen and Dutra (30) were of the opinion that the specific renal lesion was not necessarily associated with hypertension, albuminuria, renal arterial and arteriolar sclerosis, uremia or the nephrotic syndrome. Further, they stated that there was no demonstrable relationship between the degree of development of intercapillary glomerulosclerosis and the duration and degree of diabetes. The nephrotic syndrome occurred in only 5 of their 79 patients with diabetes mellitus and a morphologically proven glomerular lesion. This paper was the only one which differed sharply from the majority of previous reports. There are insufficient details in their paper to account for this marked discrepancy.

EXPERIMENTAL PRODUCTION OF DIABETIC GLOMERULOSCLEROSIS

Lukens and Dohan (35) reported experimental and autopsy observations, over a 5 year period, on a dog in whom diabetes was produced by injections of a pituitary extract. The diabetes was found to be of constant severity after the first year. At autopsy, the predominant findings were atrophy of the Islands of Langerhans, fatty deposits in the liver, and glomerular and tubular lesions which the authors considered to be similar to diabetic glomerulosclerosis occurring in humans. The glomeruli had small focal hyaline deposits in the capillary wall, but the predominating feature was the striking focal cellular proliferation, most conspicuous around the afferent arterioles. No leucocytes or other evidence of inflammation were noted. The tubules were the site of extreme patchy, fatty infiltration. The type of fat, neutral or doubly refractile, was not stated. The larger vessels and collecting tubules were not remarkable. No special stains for amyloid were performed. The eyes were found to be normal. Lukens and Dohan (35) pointed out that this was the first example known to them of "intercapillary glomerulosclerosis" occurring in an animal. They further stated that this lesion was distinct from the chronic interstitial nephritis which may occur spontaneously in dogs.

Loeb (34) stated that intercapillary glomerulosclerosis had been induced in his laboratory in rats made diabetic with alloxan.

METHODS AND MATERIAL

The diagnosis of diabetic glomerulosclerosis was clinically suspected in 45 patients of the Montefiore Hospital, during the period from 1943 to 1948. Twenty-two of these patients came to autopsy.

The diagnosis of diabetes mellitus was made from fasting blood sugar values, glycosuria, and in a few cases, by glucose tolerance tests, after adequate preparation with high carbohydrate and low fat diets.

The degree of diabetes was classified into three groups: mild, when the patient was controlled with 0-10 units of insulin in a 24 hour period; moderate, when the need for insulin was 11 to 30 units; severe, when more than 30 units of insulin for 24 hours was required.

Systolic hypertension was considered to be present if the systolic pressure was 150 mm. Hg or more. When there was no blood pressure elevation, repeated electrocardiograms were taken to determine recent or old myocardial infarction. In addition, post mortem weights of the heart over 450 grams in a male, and 400 grams in a female, were accepted as evidence of pre-existing hypertension.

The presence of the nephrotic syndrome was based on the degree and location of edema in the absence of cardiac failure, the qualitative and quantitative albuminuria, the degree of hypoproteinemia and hypoalbuminemia, the presence of doubly refractile lipid cells or casts in the urinary sediment and the paucity of red blood cells, with absent red blood cell and hemoglobin casts in the urine.

Renal insufficiency with uremia was based on the urea clearance and creatinine values. The blood urea nitrogen values were available but care was taken in their interpretation since it is known that they also depend, to a great extent, upon the degree of hydration, protein intake and extra-renal azotemia associated with cardiac failure. Uremic levels were considered to be present when the urea clearance was below 30% and creatinine values above 3 mg. %.

Analysis of the pathologic material is based on the 22 patients who came to autopsy. Sections of kidney, liver, spleen, pancreas, adrenals, myocardium and lungs were examined. At the time of post mortem examinations, tissues were fixed in formalin and in either Orth's or Zenker-Formol solutions. The original blocks and the Zenker or Orth's fixed tissues were available in all cases. Formalin fixed material was not available in all cases, and hence investigations of the distribution of lipoids and of doubly refractile substances were made in only a selected group of cases. Organ weights and descriptions were obtained from recorded protocols.

Lesions characteristic of intercapillary glomerulosclerosis were identifiable in hematoxylin and eosin stained sections. Special stains including Azan-Carmine, Masson, Foot stains for reticulum and Van Gieson stains were used when necessary. Frozen sections were stained for amyloid and fat and examined unstained for doubly refractile substances.

ANALYSIS OF CLINICAL MATERIAL

Although there has been a change in the original concept of diabetic glomerulosclerosis, the majority of reports favor the association of a marked degree of

intercapillary glomerulosclerosis with albuminuria and hypertension, and in a number of cases with a fully developed nephrotic syndrome (4, 5, 9, 11, 13, 20, 21, 23, 29, 38, 40, 43). It should be pointed out that the causative lesion in this nephrotic syndrome appears to be located in the glomerulus. The tubules have not received much attention. Some have claimed that they are normal or contain variable amounts of fat. In the cases originally described, there was striking deposition of fat and doubly refractile lipid in the tubules and in the interstitial tissues (29).

It has been known for a long time now that the urinary sediment from patients with a nephrotic syndrome due either to subacute or chronic glomerulonephritis, lipid nephrosis, renal amyloidosis, thrombosis of the renal veins, or luteal nephrosis, will show doubly refractile lipid droplets (14, 18, 32, 39). Since the nephrotic syndrome, partial or complete, has been described with diabetic glomerulosclerosis, the urines from all clinically suspected patients in Montefiore Hospital since the early part of 1943, have been specifically examined for the presence of these doubly refractile lipoids.

The diagnosis of diabetic glomerulosclerosis has been clinically suspected in 45 patients during the period from 1943 to 1948. Twenty-two of these patients have come to autopsy where the diagnosis has been confirmed in all but one case. It has been possible to demonstrate doubly refractile fatty cells or casts in association with moderate to severe albuminuria in 40 of the 45 clinically diagnosed cases. In the remaining 5 cases, no fatty cells or casts were noted in the urinary sediment, although the clinical picture was characteristic. The typical lesions of diabetic glomerulosclerosis, however, were found in all 5 cases at autopsy. Only one patient in whom the urinary sediment was strongly positive for anisotropic lipid on repeated occasions, revealed no evidence of glomerulosclerosis at autopsy. The anatomical diagnosis was moderately advanced arterial and arteriolar nephrosclerosis. We consider this case to be our only "false positive". We shall have more to say about this later on. Our clinical analysis is based on the remaining 44 patients.

Although there have been large groups of cases analyzed in the literature from autopsy protocols, we believe this series to be the largest one in which the diagnosis of diabetic glomerulosclerosis was made during life. It should be further pointed out that by the time the patient has arrived at Montefiore Hospital, he has usually traveled through the hospital circuit and is frequently in an advanced stage of his disease. We feel, therefore, that our particular series of patients represent diabetic glomerulosclerosis in its fully developed clinical state.

A. Age and sex incidence

The greatest percentage of our cases fell in the 6th and 7th decades of life. The youngest patient was 25 years of age. The oldest patient was 73 years. We had 3 patients who were in the 3rd decade. Two of these patients are still alive, and the possibility still exists that the essential lesion here is glomerulonephritis rather than glomerulosclerosis. The great number of reported patients

scribed two types of glomerular lesions characteristic of diabetes, the nodular and diffuse. The nodular lesion corresponds with the type described by Kimmelstiel and Wilson (29). Bell (7) concluded that the diffuse form of "intercapillary" sclerosis, although noted in diabetes, is much more frequent in glomerulonephritis. The spherical hyaline lesions were found only in cases of diabetes.

The clinical syndrome has undergone modification since its original description. Anson (4) found that the typical nephrotic syndrome was absent in 4 of 6 patients. He concluded that nephrosis does not seem to be an essential clinical feature. Newburger and Peters (40), on reviewing 4 of their own cases, together with the published case reports of Kimmelstiel and Wilson (29), and Anson (4), found that mild diabetes, hypertension, albuminuria and retinal vascular changes were the most prominent manifestations. They stated that although nephrotic edema was a common finding, edema associated with cardiac failure was present in 25% of their cases. This was in general agreement with Porter and Walker's (43) findings. Siegel and Allen (45) found a good correlation of the pathologic lesion with the nephrotic syndrome, but this depended chiefly on the severity of the glomerular lesion. They felt that the specific renal lesion should be diagnosed clinically when the syndrome of hypertension, azotemia, edema and hypoproteinemia and hypercholesterolemia occurs in the diabetic. They pointed out, however, that a number of their patients with the specific glomerular lesion revealed no evidence of renal damage or hypertension during life. Weiss (50) stated that intercapillary glomerulosclerosis assumed clinical interest only if the lesions were prominent and diffuse, and only then were they to be associated with albuminuria, hypoproteinemia and a tendency to anasarca.

Goodof (20) stated that the clinical diagnosis of this syndrome was justified under these circumstances—in a middle-aged patient with mild or moderately severe diabetes, having the disease for six years or more, excreting moderate to large amounts of albumin in the urine and without evidence of other renal disease.

Lefebvre and Dechard (31) analyzed seven patients with the specific lesion in the glomeruli. In this group, edema was not prominent, occurring in only two of their patients, and the cause of the edema was cardiac failure. They stated that proteinuria, arteriosclerosis, hypertension, hypoproteinemia in middle-aged individuals with mild diabetes, may be associated with intercapillary glomerulosclerosis, but such features have also occurred in persons who fail to show these morphologic features in their kidneys at autopsy. They made the point that many diabetic patients proven to have the glomerular lesion at autopsy, including some with the advanced changes, presented no characteristic clinical signs to differentiate them from diabetic patients without the specific renal lesion.

Henderson, Sprague and Wagener (23) felt that the diagnosis of intercapillary glomerulosclerosis could not be established with complete certainty during life, but should be strongly suspected in patients who have diabetes mellitus of long standing, associated with albuminuria, hypertension, renal insufficiency, mixed vascular and diabetic retinopathy. In rare instances, they found the condition associated with only diabetes and albuminuria. They emphasized the frequent association of the combined retinopathy with the glomerular lesion.

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were also in the 6th and 7th decades. Laipply, Eitzen and Dutra (30) reported the youngest patient to date, a young girl, 16 years of age, with a clinical course which was characteristic of juvenile diabetes. At post mortem examination, typical lesions of glomerulosclerosis were noted. The case is particularly important since the glomerulosclerosis was not associated with renal arteriolar-sclerosis.

In our material, females showed a somewhat greater incidence than males. It should be noted that, in Massachusetts, Joslin (27, 28) found, in the years between 1933 and 1938, a preponderance of females in diabetics in the 6th and

TABLE 1
Duration of known diabetes

NO. OF YEARS	NO.	PER CENT
1-5	7	16.1
6-10	9	18.4
11-15	16	37.9
16-20	8	18.4
20 or more	0	0
No known history	4	9.2

TABLE 2
Intensity of diabetes

	NO.	PER CENT
Mild.....	12	27.6
Moderate.....	26	58.6
Severe.....	6	13.8

7th decades. The majority of the reports were in good agreement with our figures.

B. Duration of known diabetes

It was not possible to state categorically any correlation between the duration of known diabetes and the onset of the specific glomerular lesion (table 1). There was an almost equal percentage of cases with a known duration of diabetes varying from 6 to 10 years and 16 to 20 years respectively. A somewhat smaller percentage (16.1%) had a known duration of diabetes from 1 to 5 years. In four of our patients, there was no known history of diabetes and the diagnosis was established only after intercapillary glomerulosclerosis was suspected.

C. Intensity of diabetes

Thirty-eight of our patients could be classified as having either mild or moderately severe diabetes (table 2). Twelve patients in this group required no insulin at any time. The remaining 26 patients never required more than 30

units of insulin. Only 6 of our patients needed more than 30 units of insulin. The 3 patients who required the greatest amount of insulin for adequate control were in the third decade of life. All the existing reports, with the exception of Laipply, stress, as we do, the mild character of the diabetes.

It was not possible in our series to correlate with any degree of accuracy the type of insulin and the length of time that insulin was used with the course of diabetes and the development of glomerulosclerosis.

D. Diagnosis of diabetes

The diagnosis of diabetes was established in 40 of our patients by a previous history of elevated fasting blood sugar and glycosuria. In one patient with no previous history of diabetes, an elevated fasting blood sugar and glycosuria were noted for the first time during his hospital stay. In the remaining three of our patients, there was no history of diabetes prior to present hospital admission. The diabetes was of such mild nature that it was necessary to perform glucose tolerance tests to confirm this diagnosis definitely. It should be pointed out that the presence of severe albuminuria and the finding of doubly refractile lipid cells in the urinary sediment in 2 of these cases were our first clues that the patient had diabetes.

E. Associated complications of diabetes

Only 3 of our patients gave a history of diabetic coma at any time during the course of their illness. The complications of diabetes included cataracts, peripheral neuropathies and peripheral vascular disease. Cataracts and vitreous opacities were noted in 19.2% and peripheral vascular diseases were noted in 31.2% of our series of patients.

F. Hypertension

Ninety-five per cent of the patients in our series had a systolic hypertension and 90% revealed a diastolic hypertension. In one patient, the blood pressure could not be ascertained because of obliteration of the pulses in the extremities due to severe arteriosclerosis. In 4 patients with diastolic levels below 90 mm. Hg, there was a history and electrocardiographic evidence of recent myocardial infarction. Two of these 4 patients had a systolic level below 150 mm. Hg.

The duration of hypertension prior to hospital admission was determined from the history given by the patient together with all obtainable past medical records (table 3). Twenty-four of the patients had hypertension existing for 5 years or less. Twenty-five patients developed hypertension following the known onset of diabetes. This varied, however, from 1 to 20 years. In 3 of the patients, it appeared that the onset of known diabetes and known hypertension was coincidental. An additional 3 patients revealed hypertension existing prior to the discovery of diabetes. In these patients there was no history or clinical evidence of a primary renal disease. It must be realized, however, that the diabetes in these cases was so mild and clinically quiescent that the disease may have existed long before its actual discovery.

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G. Fundoscopic findings

It became immediately apparent (table 4) that 30 of our patients presented a combined hypertensive and diabetic retinopathy, in which hemorrhages and both yellow and white exudates were present. The hemorrhages varied some-

TABLE 3
Relation of onset of hypertension to onset of diabetes

YEARS	NO.	PER CENT
Prior to onset of diabetes		
0-5	3	6.9
6-10	0	0
11-15	0	0
16-20	0	0
Coincidental	3	6.9
Following onset of diabetes		
0-5	9	20.7
6-10	3	6.9
11-15	12	29.9
16-20	1	2.3
Unknown	13	26.4

TABLE 4
Fundi

	NO.	PER CENT
1. Diabetic retinopathy, hemorrhages only.....	0	0
2. Diabetic retinopathy, hemorrhages and exudates.....	5	11.5
3. Hypertensive retinopathy		
Grade 1.....	1	2.3
2.....	1	2.3
3.....	0	0
4. Diabetic and hypertensive retinopathy, hemorrhages only..	0	0
5. Diabetic and hypertensive retinopathy, hemorrhages and exudates.....	30	67.8
6. Retinitis proliferans.....	1	2.3
7. Fundus not visualized.....	4	9.2
8. Unknown.....	1	2.3
9. Normal.....	1	2.3

what in appearance. In many cases, they were typically superficial or flame-shaped; in others, they were deep, punctate, round or oval and discrete. Frequently, these two types were seen in the same fundus. The yellow exudates were the typical lipid appearing masses seen in diabetes. It should be noted that 11.5% of our patients revealed no evidence of hypertensive retinopathy and the fundi revealed only a diabetic retinopathy with hemorrhages and exu-

dates. Two patients had a mild hypertensive retinopathy with no change suggestive of diabetes. It was impossible to visualize the fundus in 4 cases because of lenticular or vitreous changes or retinitis proliferans. One patient, age 52, presented a completely normal fundus.

H. Edema

Thirty-nine of the 44 patients presented themselves with varying degrees of edema. Of this group, 28 were in congestive heart failure. The remaining 11 patients with edema gave no clinical evidence in the past, or during the present hospital admission, of cardiac failure. The edema in these 11 patients was chiefly confined to the lower part of the body involving the legs, thighs, serotum and abdominal wall. Two of these 11 patients, in the 3rd decade of life, revealed facial edema, chiefly of the upper lids. None in this group without heart failure showed clinical evidence of pleural, pericardial, or peritoneal effusions. It should be remembered, however, that all of these patients were on the Montefiore Hospital diet which contains only 1.2 gms. of salt per day. Total plasma proteins, albumin and globulin levels were determined in 9 of these 11 patients, having edema uncomplicated by heart failure. Only 3 of these patients presented albumin levels below 2.5 gms. %. These 3 patients were the only ones in the entire group who manifested a marked degree of generalized edema. Total protein content in a 24 hour urine output was also known in these 9 patients. The proteinuria varied from 4 to 13 gms. per 24 hours. Estimations were carried out at least twice on each of these patients. No correlation could be drawn in this group of 11 patients, between the total plasma protein, albumin levels and 24 hour urinary proteins.

In an attempt to determine whether there was a coexistence of nephrotic edema, and edema associated with cardiac failure, plasma albumin levels and the degree of proteinuria were also analyzed in the 28 patients with known congestive heart failure. Six of these 28 patients revealed plasma albumin levels below 3 gms. %. Five of these 28 patients excreted as much as 19 to 20 gms. of protein in 24 hours. These were among the highest values obtained in the entire series. Again there was no correlation between the high urinary protein content and the plasma albumin level. For example, one patient spilled between 18 to 20 gms. of protein in 24 hours, estimated on 3 different occasions, but had plasma albumin levels varying between 3.4 and 3.6 gms. %.

I. Urinary sediment

We have studied the urinary sediments in all our patients with special reference to the presence of doubly refractile lipid cells or casts. As previously stated, they were found in 39 of our group of 44 patients. A review of the existing literature on diabetic glomerulosclerosis indicates that in only one paper by Derow, Altschule and Schlesinger (13), was any mention made of this important urinary finding.

Munk (39), in 1913, first described the presence of these doubly refractile lipid cells or casts in the urinary sediments of patients with lipid nephrosis.

Leiter (32) stated that these doubly refractile lipid bodies are usually present in the urine during the edematous oliguric stages of lipid nephrosis. These cellular elements have also been found in glomerulonephritis, renal amyloidosis, luetic nephrosis, thrombosis of the renal veins, and occasionally in periarteritis nodosa and visceral lupus erythematosus (14, 18, 32, 39).

These fatty droplets are usually present in epithelial cells, occasionally in white blood cells, and are frequently in the form of casts. Viewed in the ordinary light, under the low power objective, they present a yellow black appearance. They vary in size and shape, but more frequently are round to oval. In polarized light with crossed planes, they present a typical and easily recognizable appearance. The polarized light is produced either by means of crossed Nicol prisms or more simply by inserting one piece of thin polaroid film into the eyepiece and another piece into the condenser of an ordinary microscope. The eye piece is rotated until crossing of the planes of polarized light produces a dark background. In this field the fatty cells and casts are seen to contain bright, achromatic Maltese crosses with dark crosslines. The axes of these crosslines rotate when one rotates the polaroid strips or Nicol prisms (see fig. 4).

The amount of these doubly refracting lipid elements varies from day to day. They may be seen in every field in some sediments, while in other sediments only the most careful and persistent search reveals fatty cells or casts. It is important to differentiate the anisotropic lipid droplets from ordinary neutral fat which may be found in a great number of conditions including simple fatty infiltration and degeneration of the kidney. Neutral fat droplets stain a deep orange with Sudan III or IV in contrast to the faint yellowish staining of anisotropic fat droplets. In a number of urinary sediments examined, it was possible to show the admixture of neutral and anisotropic lipid fat in the same cell or cast. It is significant that the anisotropic fatty cells have been found chiefly in fresh acid urine. They are poorly preserved and difficult to find in alkaline urine.

Another important feature of the urinary sediments of our patients was the paucity of red blood cells. In 58% of our cases, no red blood cells were found, and in 42% no more than 1 to 5 red blood cells per high power field were noted. There was never any evidence of hemoglobin casts, while hyaline and granular casts were found in variable numbers.

Analysis reveals that of the 44 patients in whom a clinical diagnosis of diabetic glomerulosclerosis was made, only 5 patients had no lipid elements present in the urinary sediments. In one of these patients, the urine was persistently alkaline. In another patient with negative urinary findings, only one urine was examined. In the remaining 3 patients, many examinations of 12 hour urine concentrates failed to reveal doubly refractile lipid cells or casts. At autopsy the lesions typical of intercapillary glomerulosclerosis were found in all 5 of these patients.

Before the presence of doubly refractile lipid elements could be established as important in the differential diagnosis of intercapillary glomerulosclerosis, a control study was indicated on urinary sediments from patients with hyperten-

sive cardiorenal disease, generalized arteriosclerosis, urinary tract infection, all of which are frequently associated with diabetes mellitus. We have made such a survey on patients in the same age group as those having diabetic glomerulosclerosis.

The method was as follows: 12 hour urinary concentrates were examined; the urines were always acid in reaction; the specimens were centrifuged twice for 10 minute periods each; 25 low power fields were examined; a polarizing light was used to further confirm the presence of anisotropic fatty cells or casts; Sudan III stains were utilized to demonstrate neutral fat. The tentative clinical diagnosis was unknown to the observer.

CHART I

MATERIAL	NO OF PATIENTS	ALBUMIN	NO OF CASES PRESENTING DOUBLY REFRACTILE FATTY CELLS OR CASTS IN THE URINE
Generalized arteriosclerosis	18	0-trace	0
Generalized arteriosclerosis and diabetes	12	0-trace	0
Hypertensive renal vascular disease	30	0-1 plus	0
Hypertensive renal vascular disease and diabetes	10	0-1 plus	0
Urinary tract infections (positive urine cultures).	10	1 plus-2 plus	0
Kimmelsteil-Wilson syndrome	10	4 plus	10
Carcinoma of prostate*	3	0-trace	0
Carcinoma of urinary bladder	1	0	0
Hypernephroma	2	0	0
Malignant hypertension	2	1 plus	0
Glomerulonephritis			
Subacute	1	4 plus	1
Chronic	4	1 plus-2 plus	2
Diffuse vascular disease	9	1 plus-2 plus	0
Multiple myeloma	4	2 plus-4 plus	0
Amyloidosis (1 with nephrotic syndrome)	3	4 plus	1
Streptomycin therapy (2-6 months duration) . .	20	0	0
Leukemia (with renal infiltration proved at autopsy)	3	0	0

* Two of these patients revealed neutral fat in the urinary sediment

From Chart I, it can be seen that none of the 70 patients with generalized arteriosclerosis and hypertensive renal vascular disease, with or without diabetes, had doubly refractile fatty cells or casts in their urinary sediments. In addition, the albuminuria in these patients rarely exceeded 1 plus. In various other conditions, such as urinary tract infections, malignant hypertension, hypernephroma, multiple myeloma, leukemia with renal infiltrates (proved at autopsy) and diffuse vascular disease, no anisotropic fatty cells were noted in the urinary sediment. Because of some reports in the literature, which stated that fatty degeneration of the kidney may occur following streptomycin therapy, we have examined 20 urinary sediments from patients who received streptomycin during a period of 2 to 6 months. These have all been negative for doubly refractile and neutral

CHART II

Relationship of edema, plasma proteins, proteinuria in the entire series of patients with diabetic glomerulosclerosis, with and without cardiac failure

PATIENT	EDEMA	TOTAL PROTEIN	PLASMA ALBUMIN LEVEL	PROTEINURIA	CONGESTIVE HEART FAILURE, + OR 0
		<i>gms./100 cc.</i>	<i>gms./100 cc.</i>	<i>gms./24 hrs. or qualitative</i>	
H. R.....	3 plus	5.4	2.7	13.5	+
F. L.....	3 plus	7.0	4.5	1 plus	+
L. M.....	2 plus	6.0	2.9	2.95	+
R. M.....	3 plus	6.4	4.2	2 plus	+
F. K.....	2 plus	6.4	4.4	2.5	+
R. H.....	2 plus	5.8	2.7	8.3	+
S. G.....	1 plus	6.1	4.4	4.9	+
N. S.....	1 plus	6.8	3.4	4 plus	+
L. G.....	2 plus	6.6	3.9	4.45	+
L. R.....	4 plus	5.9	3.9	1.9	+
I. C.....	0	6.4	4.5	trace	0
F. D.....	4 plus	—	—	4 plus	+
M. R.....	2 plus	5.7	2.4	22.2	+
S. S.....	2 plus	5.5	3.3	3.69	+
M. K.....	1 plus	5.2	2.9	3.59	0
A. C.....	2 plus	5.9	3.6	19.6	+
V. F.....	2 plus	6.1	3.4	3.1	+
B. H.....	2 plus	5.5	3.5	4.2	0
J. F.....	1 plus	5.2	3.1	4 plus	0
D. R.....	1 plus	—	—	4.5	+
R. K.....	4 plus	4.9	2.6	4 plus	+
S. M.....	1 plus	7.2	5.1	7.89	0
L. S.....	2 plus	7.5	3.4	1.3	0
S. M.....	0	—	—	3 plus	0
C. H.....	1 plus	6.7	3.9	8.4	0
E. F.....	3 plus	—	—	4 plus	0
F. W.....	3 plus	7.0	4.8	trace	+
I. L.....	4 plus	6.6	3.9	2.36	+
B. D.....	1 plus	—	—	3 plus	0
D. B.....	3 plus	7.2	4.1	4 plus	+
W. B.....	2 plus	5.6	3.2	4 plus	+
I. Y.....	1 plus	6.5	3.5	8.5	+
O. S.....	1 plus	4.5	2.19	4 plus	+
S. S.....	2 plus	6.1	4.1	4 plus	+
R. S.....	3 plus	3.1	2.1	8.0	0
S. C.....	2 plus	—	—	3 plus	0
L. R.....	3 plus	5.6	3.5	4 plus	+
M. T.....	2 plus	—	—	4 plus	+
H. F.....	4 plus	6.6	4.6	4 plus	+
H. N.....	3 plus	6.5	3.9	0.73	+
A. P.....	1 plus	6.0	3.5	2.46	+
S. F.....	3 plus	4.2	2.4	6.54	0
S. C.....	0	5.9	3.3	2 plus	0
J. K.....	3 plus	5.8	3.3	4 plus	+

CHART III

A clinico-pathologic correlation of the twenty-one patients who came to autopsy

PATIENT	SEVERITY OF DIABETES	INTENSITY OF HYPERTENSION	FUNDI	PLASMA ALBUMIN gms /100 cc.	PROTEINURIA gms /24 hrs	BGR gms /100 cc
M. R.	mild	230/110	D&H*	2.4	22.2	7.5
L. M.	moderate	205/110	D&H	2.9	2.95	8.0
N. S.	moderate	230/110	D&H	3.4	4+	6.5
I. C.	mild	170/105	D&H	4.5	trace	11.5
H. R.	moderate	181/90	D&H	2.7	13.5	10.0
F. K.	moderate	160/108	D&H	4.4	2.5	10.5
L. G.	moderate	245/120	D&H	3.9	4.5	12.0
R. H.	mild	150/70	Cataract	2.7	4+	8.9
R. M.	moderate	120/80	D&H	4.2	2+	14.5
F. D.	mild	140/95	D†	—	4+	14.0
F. L.	moderate	120/60	Not visualized	4.5	trace-1+	1.5
L. R.	moderate	230/140	D&H	3.9	1.00	7.5
S. S.	mild	230/124	D&H	3.3	3.69	9.0
S. G.	severe	210/110	D&H	4.4	4.86	6.8
M. T.	moderate	200/96	D&H	—	4+	11.5
H. F.	moderate	185/90	D&H	4.6	4+	11.7
H. N.	mild	210/110	D&H	3.9	0.73	10.0
A. P.	moderate	210/110	D&H	3.5	2.46	10.5
J. K.	severe	250/180	D&H	3.3	7.0	9.0
S. F.	severe	160/90	D&H	2.4	6.54	9.0
S. C.	moderate	180/88	D&H	3.3	2+	10.0

* Diabetic and hypertensive retinopathy.

† Diabetic retinopathy.

PATIENT	BLOOD CREA- TININE	CARDIAC FAILURE	CAUSE OF DEATH	STATE OF NUTRITION	HEART WEIGHT
M. R.	2.9	+	Uremia	well nourished	490
L. M.	4.9	+	Uremia	well nourished	530
N. S.	9.8	+	Uremia	well nourished	450
I. C.	—	0	Acute heart failure following coronary occlusion	obese	580
H. R.	—	+	Uremia	well nourished	480
F. K.	3.5	+	Cardiac failure	obese	470
L. G.	6.4	+	Uremia	poorly nourished	550
R. H.	1.0	+	Cardiac failure	poorly nourished	400
R. M.	—	+	Cardiac failure	obese	410
F. D.	3.2	+	Cardiac failure	obese	500
F. L.	—	+	Cardiac failure	markedly obese	420
L. R.	9.5	+	Uremia	well nourished	560
S. S.	4.2	+	Cardiac failure and renal insufficiency	obese	458
S. G.	13.0	+	Uremia	obese	500
M. T.	3.1	+	Bronchopneumonia	well nourished	400
H. F.	—	+	CA. of rectum	obese	700
H. N.	—	+	Cardiac failure	poorly nourished	200
A. P.	3.3	+	Uremia	poorly nourished	350
J. K.	8.4	+	Uremia	well nourished	410
S. F.	1.8	0	Myocardial infarct	poorly nourished	400
S. C.	3.3	0	Multiple myeloma	poorly nourished	290

CHART III—Continued

PATIENT	MYOCARDIAL INFARCTION	WEIGHT OF KIDNEYS		DEGREE OF INTERCAPILLARY GLOMERULO-SCLEROSIS	DEGREE OF CHANGE IN ARTERIOLES
		Rt.	Lt.		
M. R.....	posterior wall, old	190	185	3+	3+
L. M.....	0	170	180	3+	3+
N. S.....	0	260	220	2+	3+
I. C.....	multiple, old & recent	250	250	2+	1+
H. R.....	0	110	150	1+	3+
F. K.....	anterior wall, old	135	130	2+	3+
L. G.....	recent, posterior wall	80	90	3+	3+
R. H.....	posterior wall, old	130	120	3+	3+
R. M.....	multiple, old and recent	250	240	1+	1+
F. D.....	0	180	200	1+	3+
F. L.....	posterior wall, septal, old	170	150	1+	3+
L. R.....	0	245	250	3+	3+
S. S.....	0	280	265	3+	3+
S. G.....	0	260	250	3+	3+
M. T.....	0	100	95	3+	3+
H. F.....	old and recent infarcts	200	220	2+	3+
H. N.....	multiple old infarcts	80	90	1+	3+
A. P.....	scar I.V. septum	90	110	3+	3+
J. K.....	0	140	160	3+	3+
S. F.....	0	180	170	1+	3+
S. C.....	0	200	190	2+	2+

PATIENT	HYALINIZATION OF ISLETS OF LANGERHANS
M. R.....	0
L. M.....	+
N. S.....	0
I. C.....	0
F. K.....	0
L. G.....	0
R. H.....	0
R. M.....	0
F. D.....	0
F. L.....	0
L. R.....	0
S. S.....	0
S. G.....	0
M. T.....	0
H. F.....	+
H. N.....	0
A. P.....	0
J. K.....	0
S. F.....	0
S. C.....	0

fatty elements. Two of 3 patients with carcinoma of the prostate had neutral fat in the urine, but intensive search failed to reveal anisotropic fatty cells or casts. Of 5 patients with glomerulonephritis, 3 had doubly refractile lipid elements in the urine. One of the patients with amyloidosis presented a nephrotic syndrome with a typical urinary sediment. In our control urines, except for the patients with glomerulonephritis, malignant hypertension, urinary tract infections and diffuse vascular disease, red blood cells were rarely found in the urinary sediment.

J. Cholesterol levels

Twenty-four of our patients revealed a total cholesterol level above 250 mgm. %. Eleven of these patients had levels which varied between 300 and 500 mgm.

TABLE 5
Urea clearances

	NO.	PER CENT
61% and greater	2	4.6
41-60%	8	18.4
11-40%	20	44.8
6-10%	3	6.9
No mention	11	25.3

TABLE 6
Blood creatinine levels

MGs./100 cc.	NO.	PER CENT
0-1.5	2	4.6
1.6-2.0	3	6.9
2.1-4.9	17	37.9
5.0 plus	9	20.7
Unknown	13	29.9

%. The highest total cholesterol level obtained was 930 mgm. %, in a patient who manifested a marked degree of nephrotic edema and whose plasma albumin level was 3.5 gms. %.

K. Renal function

Twenty patients had urea clearances below 40% and 29 patients revealed creatinine levels above 1.5 mg. % (tables 5, 6, 7). Renal hemodynamics were studied in one of our patients. This patient revealed a glomerular filtration rate reduced to 52 cc. per minute and a reduced renal plasma flow of 226 cc. per minute with an elevated filtration fraction of 23%. Coreoran, Taylor and Page (12) found similar results in their study of 6 patients with intercapillary glomerulosclerosis. They noted a reduction in GFR, Tmp, FF, and an increase in the ratio RPF/Tmp. The mechanism of the change was apparently similar to that of glo-

CHART III—*Continued*

PATIENT	MYOCARDIAL INFARCTION	WEIGHT OF KIDNEYS		DEGREE OF INTERCAPILLARY GLOMERULO-SCLEROSIS	DEGREE OF CHANGE IN ARTERIOLES
		Rt.	Lt.		
M. R.....	posterior wall, old	190	185	3+	3+
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F. K.....	anterior wall, old	135	130	2+	3+
L. G.....	recent, posterior wall	80	90	3+	3+
R. H.....	posterior wall, old	130	120	3+	3+
R. M.....	multiple, old and recent	250	240	1+	1+
F. D.....	0	180	200	1+	3+
F. L.....	posterior wall, septal, old	170	150	1+	3+
L. R.....	0	245	250	3+	3+
S. S.....	0	280	265	3+	3+
S. G.....	0	260	250	3+	3+
M. T.....	0	100	95	3+	3+
H. F.....	old and recent infarcts	200	220	2+	3+
H. N.....	multiple old infarcts	80	90	1+	3+
A. P.....	scar I.V. septum	90	110	3+	3+
J. K.....	0	140	160	3+	3+
S. F.....	0	180	170	1+	3+
S. C.....	0	200	190	2+	2+

PATIENT	HYALINIZATION OF ISLETS OF LANOERHANS
M. R.....	0
L. M.....	+
N. S.....	0
I. C.....	0
F. K.....	0
L. G.....	0
R. H.....	0
R. M.....	0
F. D.....	0
F. L.....	0
L. R.....	0
S. S.....	0
S. G.....	0
M. T.....	0
H. F.....	+
H. N.....	0
A. P.....	0
J. K.....	0
S. F.....	0
S. C.....	0

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Unknown	13	29.9

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merulonephritis. The pattern was one of decreased filtration presumably due to lesions of the glomerular capillaries associated with a loss of tubular secretory function. In our patient, there was only a mild glomerulosclerosis but extensive arteriolar nephrosclerosis. The elevated filtration fraction was probably due to the efferent arteriolar constriction. From Page's data, it is apparent that there was a wide range of variation in the filtration rate and renal plasma flows. One must consider the effect both of arteriolar and glomerular capillary involvement in these cases of diabetic glomerulosclerosis.

TABLE 7
Blood urea nitrogen levels

MGS./100 cc.	NO.	PER CENT
1-15	0	0
16-30	13	29.9
31-50	9	20.7
50 plus	21	47.1
No mention	1	2.3

TABLE 8
Hemoglobin levels

AMOUNT <i>gms./100 cc.</i>	NO.	PER CENT
14.1-15	3	6.9
13.1-14	2	4.6
12.1-13	2	4.6
11.1-12	15	33.3
10.1-11	5	11.5
9.1-10	6	13.8
Less than 9.1	11	25.3

L. Anemia

Twenty-two of our patients had hemoglobin levels below 11 gm. % (table 8). Eleven of these patients had a moderately severe anemia as indicated by levels below 9 gm. %. Nineteen of these patients had red blood cell counts below 3.5 million per cc. (table 9). The color indices were calculated and the general range varied from 0.9 to 1.1. There were 5 cases with color indices which varied from 0.80 to 0.88. The anemia appeared to be definitely related to the degree of renal insufficiency, but malnutrition certainly had played a part in the production of the anemia.

M. Cause of death

Nine of the 22 patients who came to autopsy died in uremia, seven patients in cardiac failure, and 2 patients because of an acute coronary occlusion. One

patient died with carcinoma of the rectum, another because of bronchopneumonia and a third patient with multiple myeloma.

PATHOLOGIC ANATOMY

The pathogenesis of diabetic glomerulosclerosis is not yet known. The site of origin of the glomerular lesion, whether intramural or intercapillary is disputed. The majority of observers are in agreement with the former concept. However, there is no disagreement with regard to the morphologic picture of the lesion in its fully developed stage. The minute histologic details have been

TABLE 9
Erythrocyte count

MILLION / CC.	NO.	PER CENT
4.6-5.0	1	9.2
1.1-4.5	7	16.1
3.6-4.0	13	28.7
3.1-3.5	10	23.0
2.6-3.0	7	16.1
2.0-2.5	2	4.6
Unknown	1	2.3

TABLE 10
Cause of death

	NO.	PER CENT
Uremia	9	42.1
Cardiac failure	7	33.6
Acute coronary occlusion	2	9.6
Cerebro-vascular accident	0	0
CA of rectum	1	4.8
Broncho-pneumonia	1	4.8
Multiple myeloma	1	4.8

abundantly described in the original communication of Kimmelstiel and Wilson (29) and subsequently in the exhaustive studies of Allen (3). The characteristic lesion consists of a spherical to oval, focal, pale to deeply staining acidophilic mass of usually acellular dense hyaline material, and occupying the center of a glomerular lobule; patent capillaries may be seen at its periphery. The lesions vary in size and number, involving few to almost all the glomeruli in a given section (fig. 1). The involved glomeruli are usually of average size or even somewhat larger than usual. Progression to various stages of complete glomerular atrophy, hyalinization, and obliteration are found with variable frequency.

The most striking finding according to some investigations is the high inci-

dence of arterial and arteriolar nephrosclerosis. Henderson and his coworkers (23) have stressed the fact that the histologic criteria on which to base a diagnosis of minimal intercapillary glomerulosclerosis are not readily defined. Others have described the earliest lesions of intercapillary glomerulosclerosis and have differentiated these lesions from the atrophic glomerular changes associated with ordinary arteriolar nephrosclerosis and senility. These have been variably described as focal fibrosis or small deeply stained, club-shaped masses situated in the midst of diffuse thickening along the axis of the lobule, or as focal irregular thickenings in the absence of appreciable generalized capillary basement membrane thickening.

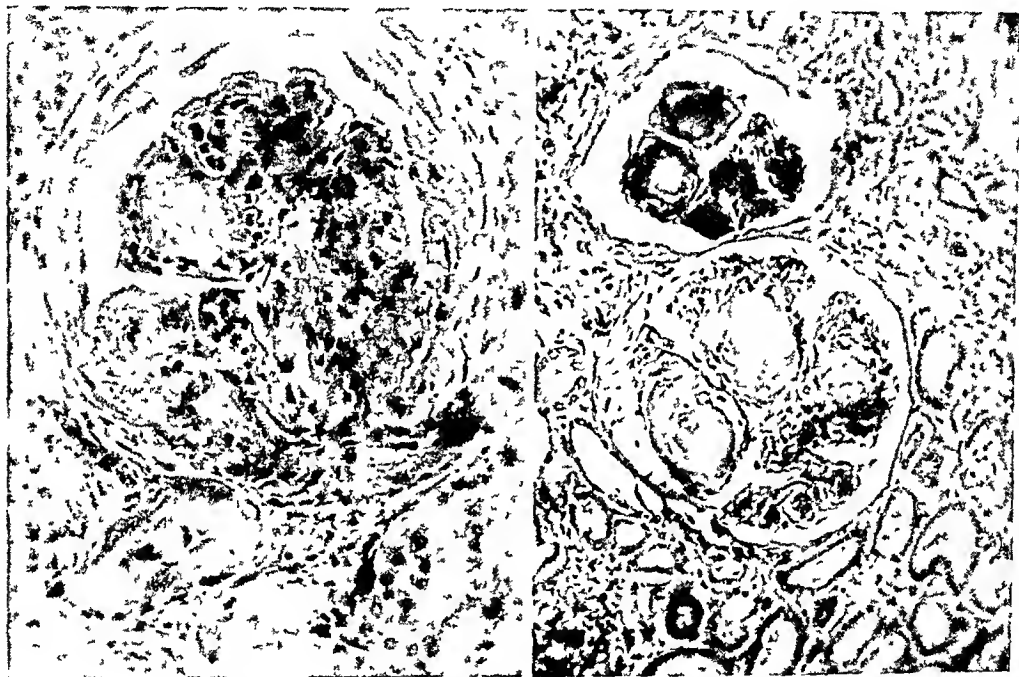


FIG. 1. The glomerulus in A (left) demonstrates a single typical hyalin ball ($\times 320$). Note the severe efferent as well as afferent arteriosclerosis. Multiple lesions of varying size are shown in B (right) ($\times 190$).

We have arbitrarily graded the specific glomerular lesion in the following manner. Intercapillary glomerulosclerosis was regarded as absent when only diffuse uniform capillary basement membrane thickening was found without irregular focal lesions (fig. 2). Focal thickenings regardless of the number of glomeruli involved, together with a rare well developed spherical lesion in any one section, was considered slight. Single or multiple fully developed lesions involving as many as 60% was designated moderate. When single, but usually multiple, unmistakable lesions were found in practically all glomeruli, the involvement was considered severe.

We have also estimated the degree of renal arteriosclerosis. Patchy and eccentric hyaline thickening of most of these vessels or the earliest recognizable diffuse concentric thickening, with no significant encroachment on the lumen, was considered slight. Diffuse concentric arteriolar hyalinization with appreci-

able luminal narrowing was graded as moderate. When there was extensive reduction of the lumen, due to massive hyalinization of the arteriolar wall, the lesion was called severe.

The degree and extent of interstitial and focal fibrosis, tubular atrophy and dilatation, and arterial sclerosis of large, medium and small intrarenal arteries were also graded as slight, moderate or severe.

The characteristic lesions of intercapillary glomerulosclerosis were found in the kidneys of 21 of the 22 cases. The severity and the degree of accompanying renal arteriolar sclerosis are shown in table 4. In one case neither well developed

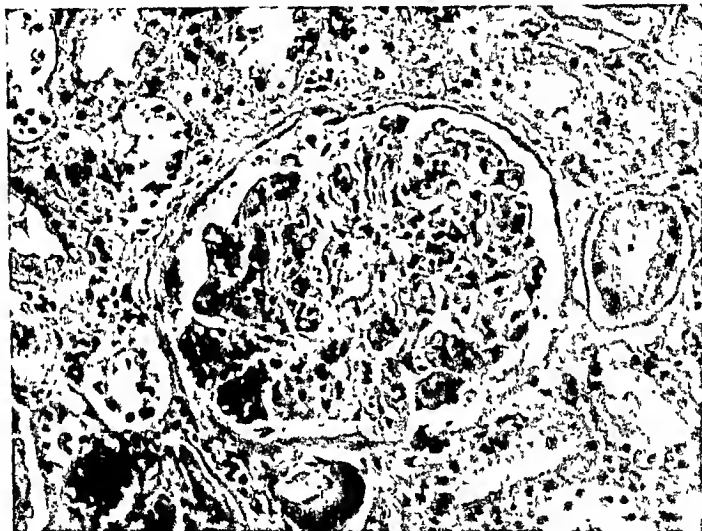


FIG 2. This glomerulus reveals diffuse capillary basement membrane thickening associated with arteriolar sclerosis. This was not considered a characteristic diabetic lesion ($\times 320$).

hyaline balls nor even focal thickenings were detectable in any sections stained with hematoxylin and eosin and Azan-Carmine. In this case, there was only a moderate arteriolar sclerosis with mild diffuse uniform glomerular capillary thickening. Almost every glomerulus was swollen and congested as were the interstitial vessels.

In one patient with multiple myeloma, one or more hyaline balls were found in slightly less than 50% of the glomeruli. In addition, there was a severe arteriolar sclerosis. The usual methods failed to confirm or exclude amyloid deposition in the spleen, liver and kidney. However, since amyloid deposits which are found in association with multiple myeloma may be atypical, and because of the similarity of the lesions of intercapillary glomerulosclerosis and

those of early glomerular amyloid deposits, we were reluctant to rule out the latter with certainty. Of perhaps greater significance was the presence of tubular lesions which may be found in the kidneys of patients with multiple myeloma alone, and generally accepted as specific for so-called "myeloma kidney" (26). While these changes are usually associated clinically with severe albuminuria, the development of hypertension or edema is exceptional, as is any significant glomerular or renal vascular alteration.

In two cases, one with slight and the other with moderate glomerulosclerosis, chronic glomerulonephritis had to be excluded. Study of multiple sections revealed few to moderate isolated adhesions between focally fibrosed glomerular loops and Bowman's capsule arranged in a patchy distribution. There were no active inflammatory or proliferative glomerular lesions. In one patient, despite the absence of gross renal contraction, there were numerous completely hyalinized glomeruli with widespread parenchymal atrophy and fibrosis resulting from massive vascular sclerosis. In another patient, with a greater number of glomerular adhesions, only focal cortical scars without significant alteration of the renal architecture, in addition to moderate arteriolar sclerosis were noted. The vascular changes of malignant nephrosclerosis were not found, in any of the 22 cases. There was no evidence in any of the sections from the 22 patients of pyelonephritis or necrotizing papillitis.

Severe atrophy and fibrosis compatible with renal insufficiency were found in 9 cases, associated with severe arterial and arteriolar nephrosclerosis. The discrepancy between the size and gross appearance of the kidneys in many instances and the morphologic evidence of extensive loss of functional nephrons with compensatory hypertrophy of remaining tubules was a striking feature (fig. 3). It is said that the average weight of kidneys is about 15% greater in well nourished than in emaciated individuals (6). There was a rough correlation between the state of nutrition and kidney weights in our cases; the larger kidneys were usually found in obese individuals.

By utilizing stained and unstained frozen sections of formalin fixed material in conjunction with polarized light, doubly refractile lipid substances were found in the tubular epithelium (fig. 4). It has been repeatedly shown that this is a non-specific finding, having been reported in many nephropathies (19). Deposits of anisotropic lipid in the tubular epithelium are ordinarily associated with a significant thickening of the basement membrane. The appearance of anisotropic lipid droplets in the urines of patients with the nephrotic syndrome, but not usually with other renal inflammatory and vascular disease, has not been adequately explained. Munk (39) has noted that these lipid cells or casts do occasionally appear in the urine of patients with very severe "arteriolar sclerotic degeneration" of the kidney.

There is but one case reported (30) in the literature in which specific lesions of intercapillary glomerulosclerosis were found in the absence of renal arteriolo-sclerosis. The authors considered it a minimal lesion. Most observers have stressed the relationship between intercapillary glomerulosclerosis and arteriolo-sclerosis. It was our impression that the intercapillary glomerulosclerosis was frequently associated with arteriolar sclerosis.

involved in this process, as pointed out by Allen (3) (fig. 1). In our cases, severe glomerular changes of a specific nature were ordinarily associated with severe hyalinization of renal arterioles with or without extensive alterations in renal architecture. However, in some cases only slight degrees of intercapillary glomerulosclerosis were associated with severe renal vascular disease. The reverse was less frequently seen. In many instances, the renal vascular disease alone was sufficient to account for the clinical findings. The significance of the superimposed unique specific lesions from a functional point of view is difficult to assess. It appears obvious, however, that arteriosclerosis alone is not sufficient to explain the origin of the diabetic glomerular lesion.

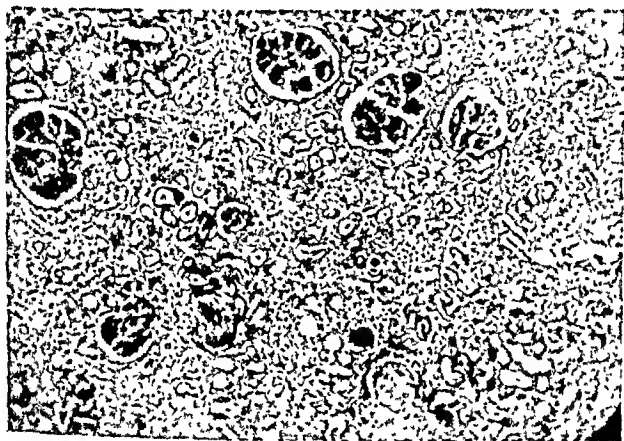


Fig. 1. Note the multiplicity of lesions involving practically all glomeruli, associated with tubular atrophy and generalized fibrosis ($\times 55$).

There is considerable controversy whether intercapillary glomerulosclerosis or hyaline changes in the pancreatic islets are found with greater frequency in patients with diabetes mellitus. We found changes in the Islands of Langerhans in only 2 of 21 patients whose kidneys showed glomerulosclerosis.

CLINICO-PATHOLOGIC ANALYSIS

A. Relation of the renal lesion to the duration of diabetes

Fourteen of the 21 patients in this group who came to autopsy had diabetes for 15 years or longer. No definite correlation could be made between the duration of diabetes and the severity of the glomerular lesion. One patient with a known history of diabetes less than one year revealed a 3 plus lesion, while 3 patients having a known history of diabetes for 3 years or longer revealed only a 1 plus intercapillary glomerulosclerosis. The diabetes, however, may be of such mild intensity that the actual duration of the disease is much longer than



FIG. 4. Aggregates of anisotropic lipids outline the renal tubules as viewed with polarized light in A (top) ($\times 320$). Maltese crosses are not distinct because of clumping of lipids. Individual Maltese crosses are well seen in B (bottom) ($\times 190$).

the known history as revealed by the patient. Eighteen of the 21 patients who came to autopsy revealed a severe degree of arterial and arteriolar nephrosclerosis. Thirteen of the 14 patients having diabetes for 15 years or longer were in this category.

B. Relation of the severity of diabetes to the renal lesion

In our series, no definite correlation was obtained between the severity of the glomerular lesion and the intensity of the diabetes. Three of the 12 patients with moderately severe diabetes revealed a 1 plus glomerular lesion, while another 5 patients with the same intensity showed a 3 plus intercapillary glomerulosclerosis. Eighteen of the 21 patients showed a 3 plus arterial and arteriolar nephrosclerosis. This included 5 patients with mild diabetes, 10 patients with moderately severe diabetes and 3 patients with severe diabetes.

C. Relation of hypertension to the renal lesion

Ten of the 21 patients of our autopsy group having a known systolic and diastolic hypertension or a history of diastolic hypertension prior to coronary occlusion, showed a 3 plus glomerular lesion, while the other 11 patients presented a milder degree of intercapillary glomerulosclerosis. However, 19 of these 21 patients with hypertension revealed a severe degree of arteriolar nephrosclerosis. The remaining 2 patients presented a 1 plus arteriolar nephrosclerosis, but there was an accompanying mild or moderate glomerular lesion. Eighteen of these patients had hearts weighing 400 grams or more. The 3 patients with hearts weighing less than 350 grams were all poorly nourished females.

D. Relation of albuminuria to the renal lesion

Seventeen of the 21 patients with varying degrees of intercapillary glomerulosclerosis revealed a 4 plus albuminuria. This group included 3 patients with a 1 plus, 4 patients with a 2 plus and 10 patients with a 3 plus glomerular lesion. No definite correlation existed between the severity of the intercapillary glomerulosclerosis and the degree of albuminuria. This was particularly noted in 2 of our patients, one of whom had a 1 plus lesion and spilled 13.5 gms. of protein in a 24 hour urine output, while another had a 3 plus glomerulosclerosis and spilled only 1.9 gms. of protein per 24 hours urine output. Qualitatively, the urines of both these patients gave a 4 plus reaction for albumin.

The question has been raised in some reports as to the exact cause of albuminuria. A number of authors have stressed the fact that in many instances the severity of arteriolar and arterial nephrosclerosis was of sufficient magnitude to produce the albuminuria. It has also been claimed that passive congestion of the kidneys secondary to cardiac failure as well as diabetic acidosis may be responsible for the albuminuria. According to Fishberg (18), albuminuria is a common finding in passive congestion of the kidney, but usually slight, amounting to less than 0.19%. In exceptional cases, especially in older individuals with nephrosclerosis, the albuminuria may be much more marked. In our

experience, a severe degree of albuminuria in cardiac failure, even with nephrosclerosis, has been rarely seen. However, it is true that 93% of the patients were in heart failure and 83% revealed a severe degree of arteriolar nephrosclerosis. As a matter of fact, one patient who spilled 13.5 gms. of protein in 24 hours, and who was in severe congestive failure, revealed a severe degree of arteriolar nephrosclerosis and only a 1 plus intercapillary glomerulosclerosis.

E. Relation of the renal lesion to uremia

Nine of our patients who came to autopsy died in uremia. These patients showed a severe degree of arterial and arteriolar sclerosis. In addition, 7 of these patients presented the most severe degree of intercapillary glomerulosclerosis.

Bell (7) states that only 4 of 606 patients (0.7%) with diabetes, uncomplicated by intercapillary glomerulosclerosis, died in uremia. Goldring and Chasis (22) have seen only one proven instance of uremic death in the course of hypertensive disease. Death due however to uremia in the acute progressive stage of hypertension, has occurred in 8% of their cases. Fishberg (18) states that uremia was the cause of death in 7% of a series of 72 cases of essential hypertension. Uremia occurred more often in diabetic patients with intercapillary glomerulosclerosis than without. Siegal and Allen (45) are in agreement with a high incidence of uremia as a cause of death in their patients.

It appears probable that the combined arterial, arteriolar and glomerular lesions are responsible for this high incidence of death due to uremia.

DISCUSSION

Results of our investigation strongly indicate that there is a recognizable clinical picture associated with the specific renal vascular complication of diabetes. It is felt that the fully developed picture is seen in a patient with long-standing diabetes, usually past the age of 50, with hypertension edema, diabetic and hypertensive retinopathy, with albuminuria and anisotropic lipid droplets in the urinary sediment. It is realized that this represents the advanced syndrome seen in diabetic glomerulosclerosis. All gradations are possible and one must be constantly on the alert when dealing with diabetics in the first decade of their disease to detect any clues of the development of intercapillary glomerulosclerosis. There are a number of factors which may distort the clinical picture at any one time. The diabetes may be so mild as to escape detection until specifically searched for by means of the glucose tolerance test. The diabetes usually is mild or moderately severe in nature, but the presence of severe diabetes should not rule out consideration of intercapillary glomerulosclerosis, especially in the younger age groups. Although the majority of our patients were over 50 years of age, it is possible to find this condition present in a younger diabetic. We are in agreement with those authors who have stressed the point that one must be sure that the patient is not suffering from chronic

glomerulonephritis. Hypertension is present in the vast majority of patients. When it is not present, myocardial infarction has usually occurred. Therefore, a normal blood pressure should not be considered a deterrent to the diagnosis of intercapillary glomerulosclerosis. Edema may or may not be present even though the remainder of the picture is fully developed. When edema is noted, it may be nephrotic, cardiac or both. The majority of our patients presented edema associated with congestive heart failure, irrespective of age. A number of these patients had a degree of hypoalbuminemia with massive proteinuria, sufficient to indicate the coexistence of nephrotic edema.

The absence of edema in a small number of these cases may have been associated with a good protein intake and low protein excretion and the absence of heart failure. It should be remembered that many of our patients would certainly have manifested a greater degree of edema, had it not been for the rigid sodium restriction of the Montefiore Hospital diet which contained 1.2 gms of salt per day.

A very important finding is the presence of a combined hypertensive and diabetic retinopathy. One may easily overlook the lipid appearing exudates unless the periphery, especially the macular area, is carefully scrutinized. These typical findings may be hidden by vitreous or lenticular opacities which themselves are often complications of diabetes.

Varying amounts of albumin are universally present in the urine. A most important aid in diagnosis of diabetic glomerulosclerosis is the presence of anisotropic lipid cells or casts. In a diabetic patient over 50, the finding of these cellular elements in the urine is usually enough to differentiate diabetic glomerulosclerosis from hypertensive renal vascular disease. It must be emphasized that a polarizing device is not essential for the recognition of these elements; that they are best preserved in fresh acid urines; that they may vary in amount from day to day, and therefore should be searched for repeatedly in 12 hour urinary concentrates. In fact, the finding of these cells in the urinary sediment in a patient in the 6th or 7th decade with hypertension and albuminuria may be the first indication to the clinician of the existence of diabetes. The associated paucity of red cells in these patients is striking. In the younger age group, doubly refractile lipid cells may be found in subacute or chronic glomerulonephritis. However, in these cases, there may be a suggestive history, and one should be particularly careful in studying urinary sediments for old red cells, red cell casts and hemoglobin casts. Anisotropic fatty cells have also been noted in renal amyloidosis. The remote possibility exists that hypertension and diabetes may be associated with this renal amyloid change, especially in patients with pulmonary tuberculosis. When fatty cells are noted in the urine, one can immediately exclude the possibility of acute glomerulonephritis.

When the correlation between the clinical and pathologic findings is made, the striking feature is the extensive renal arteriolar sclerosis, rather than the relation exists between hypertension and arteriolar nephrosclerosis, rather than with intercapillary glomerulosclerosis. Analysis reveals no definite correlation

between the degree of albuminuria and the severity of the intercapillary glomerulosclerosis. It should be realized that the functional permeability of the glomerular capillaries cannot be ascertained from the study of the histologic lesion.

One is impressed with the large group of our patients who have died in uremia, which is not a frequent cause of death in patients with benign hypertensive renal disease or uncomplicated diabetes. This is believed to be due to the combined arteriolar and glomerular capillary involvement.

We have found a relatively high number of patients with anemia. This appears to be related to the degree of renal insufficiency, although the question of nutrition must be considered. In contradistinction to previous reports, the anemia cannot be used as an aid in the differential diagnosis of intercapillary glomerulosclerosis from other renal diseases.

We are aware that lesions typical of intercapillary glomerulosclerosis have been and will be found in diabetic patients who during life apparently presented none of the clinical features described here. This disparity between clinical and pathologic findings may be ascribed to several factors: the patient may have died too early in the course of his disease; the diabetes may have been so mild as to escape notice; the apparent lack of hypertension may have been due to myocardial infarction; edema may not have been present or ascribed to cardiac failure alone; urines may not have been adequately examined for albumin and anisotropic lipid cells.

Reports have appeared indicating the presence of diabetes, hypertension and edema with no evidence of intercapillary glomerulosclerosis at post mortem examination. These authors have pointed out that the edema was due to congestive heart failure, and the hypertension was associated with arteriolar nephrosclerosis. It is in such cases that the findings of doubly refractile lipid cells or casts is an important differential diagnostic aid.

It is felt that the status of diabetic glomerulosclerosis as a clinical entity will be crystallized, once criteria for diagnosis are recognized, especially since diabetics continue to live on for a longer period of time with their disease. We have described the fully developed syndrome of diabetic glomerulosclerosis. Since the diabetic is under constant scrutiny, it is the problem of the physician in his office, the out-patient department or hospital, to be at all times aware of this vascular complication of diabetes mellitus. The finding of anisotropic lipoids in the urinary sediment establishes the diagnosis.

SUMMARY AND CONCLUSIONS

1. A series of 45 patients were diagnosed during life as having diabetic glomerulosclerosis. 22 patients came to autopsy, and 21 revealed histologic evidence of the specific glomerular lesion.

2. A distinct clinical picture is associated with diabetic glomerulosclerosis. This picture includes diabetes, edema, hypertension, combined diabetic and hypertensive retinopathy, albuminuria and lipid cells in the urinary sediment. Variants of this syndrome are discussed.

3. The development of the clinical syndrome is related to the combined effects of renal arteriolar and glomerular capillary lesions.

4. The presence of doubly refractile fatty cells or casts in the urinary sediment establishes aid in the diagnosis of diabetic glomerulosclerosis, provided it is properly related to other clinical data.

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